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THE OVOGENESIS OF HYDRA FUSCA — A PRELIMINARY PAPER.

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Kleinenberg in his thesis on hydra, 1872, maintained the following twelve points in regard to the formation of the ovary and the growth of the egg:

1. The ovary is formed by a rapid multiplication of the interstitial cells at the site of the ovary.

2. There is evidence in the relative infrequency of the interstitial cells, in the territory about the forming ovary, that their accumulation may be, in part, due to migration, though he has no direct evidence of this.

3. The interstitial cells at the center of the ovary are larger than those at the margin.

4. The interstitial cells are arranged in rows converging toward the center of the ovary.

5. The egg appears in the midst of the cells of the ovary where it has likely lain, indistinguishable from the interstitials.

6. It now comes to be distinct because of its rapidly increasing size and its irregular contour.

7. Later it becomes amœboid.

8. In the early stages of the egg granules appear in its substance which are equivalent to the white of the egg (protagon) of the eggs of higher forms.

9. These disappear and then the egg gradually fills up with the so-called "Pseudozellen" or yolk granules.

10. The cells about the egg in the ovary disintegrate to form nutritive material for the egg.

11. The egg thus increases greatly in size and with the mass of interstitials about it, greatly distends the ectoderm cells which elongate into fibers and are crowded to one side.

12. The egg breaks through these restraining ectoderm cells which are transformed into lamellæ at their peripheral end but are still connected with the muscle layer of the mesoglœa.

13. Brauer [1] describes the newly extruded egg as spherical, except for a stalk which attaches the egg to the parent. There is, too, a conical depression in the peripheral layer of the egg which layer is free from yolk. This depression lies opposite the attaching stalk over the nucleus, and by it the sperm has access.

14. Brauer states that the hydra egg gives off two polar bodies.

In attempting to confirm these results on sections of hydra prepared while working on another hydra problem, evidence accumulated that seemed to contradict some of these results. Additional material now convinces me that some of these statements are incorrect for *H. fusca* and permits me also to settle some additional points, notably on the chemical nature and the exact method of the inclusion of the yolk.

1. The multiplication of interstitial cells at the site of the ovary is by mitosis, twelve chromosomes appearing in the figure.

2. The interstitials are no less frequent immediately about the ovary than elsewhere, so there is no evidence of their migration into the region of the ovary.

3. The cells at the center of the ovary are larger than those at the margin, as Kleinenberg states. My measurements show that the central cells average four times, or a trifle more, the volume of the marginal. It is to be remembered that these central cells are adjacent to the growing eggs. The influences causing growth seem to operate on the whole region.

4. Kleinenberg, working largely on *H. viridis*, and later authors, notably R. Hertwig, working on *H. fusca*, claim that the egg appears only after the ovary has achieved considerable size. My sections seem to force the conclusion, however, that the egg is always present, before proliferation of the interstitials begins to form the ovary. The egg is often, and so far as I can see always, growing rapidly before the interstitials begin to multiply. It

[1] *Zeit. f. wiss. Zool.*, V., 52, pp. 167–216.

looks, therefore, as if the increase in size of the egg or eggs might be the cause or at least the occasion of the multiplication of these insterstitial cells.

5. R. Hertwig[1] and others, maintain that the egg is merely an interstitial cell which, after the ovary has begun to grow, increases in size more rapidly than its fellows. The egg seems in my sections always recognizable as such in the adult hydra. It is slightly larger than the inactive interstitials, has a very large nucleus in proportion to the cell body, adjacent to which there lies in this early stage a small dark ovoid body. The cell outline is spherical, whereas the resting interstitials are polygonal in section. There often appears at the early stage and always a little later, as Kleinenberg pointed out, a vacuole near the nucleus. All gradations from the large undoubted egg to this cell are readily found. But intermediates between it and the interstitials are, I may not yet say, wanting, but certainly rare. The evidence seems to point then to distinct germ cells in the adult hydra.

6. My results, furthermore, disagree with Kleinenberg's interpretation of the nutrition of the egg and extend the observations on the origin of the yolk granules as follows: At first the egg is nourished as are the adjacent ectoderm and interstitial cells. Material absorbed by the endoderm cells is massed in spherules filled with brown droplets, perhaps granules. This is apparently transformed, in large measure, in the endoderm cells and passed to the ectoderm, as this brown material seldom appears in the ectoderm cells. The endoderm cells elaborate also a material which stains deeply with osmic acid. This is also passed to the ectoderm, the cells of which are more heavily laden with it than are the endoderm cells. It passes into the egg and interstitial cells as well as others. The usual lecithin tests show it to be that substance or a closely related one. The egg at first contains the lecithin diffuse, but later in granular masses, "Pseudozellen." The interstitial cells also absorb it and the nuclei become filled with it, meantime enlarging considerably. Those interstitial cells adjacent to the egg in the fairly mature ovary have their walls in contact with the egg resorbed and the content of the cell becomes

[1] "Über Knospung und Geschlechtsentwicklung von *H. fusca*," *Biol. Centralbl.*, Vol. 26, 1906.

part of the egg (Nussbaum). The greatly enlarged nuclei, gorged with lecithin, also become yolk granules or "Pseudozellen."

7. Two polar bodies are extruded by the egg, as other observers have noted. The material thus far examined will not permit a final statement as to the details of reduction, but the first polar body contains twelve chromosomes, the second six. Certainly the female pronucleus has six, as has the male pronucleus also.

8. The nucleus and chromosomes though varying greatly in size are constantly present, never disintegrating and disappearing.

NOTES ON THE IDENTIFICATION OF THE CHÆTOGNATHA.

E. LE ROY MICHAEL.

Introductory Remarks.

It is the testimony of all, who have attempted the identification of the Chætognatha, that, for so small a group, they offer an immense amount of difficulty. The several species are very similar in appearance so that one is compelled to seek among details of structure for valid taxonomic characters. There is, moreover, considerable variation in most of the characters; some of the valuable diagnostic features are readily destroyed even with the best possible preservation, and the methods of microscopical technique, usually so efficient, fail, for the most part, with the Chætognatha. It is not surprising then, that, without most careful examination, it becomes easy to base identifications on abnormalities or upon too variable characters. This has been repeatedly done by the earlier investigators with the result that the various species have become so entangled in the literature that, for anyone not a specialist on the group, identification is well nigh an impossible task. To meet this need for an adequate and ready means of identification the keys and tables herein presented have been prepared.

Except in the papers of Fowler ('05, '06), Hertwig ('80), and Krumbach ('03) identification has been based upon characters having little or no taxonomic value. Hertwig's investigations are clear and to the point but, unfortunately, only a few species came under his observations. Krumbach has accurately made a detailed study of the seizing jaws and has developed a classification based upon the minute anatomy of these structures. It is an exceedingly valuable contribution for, in many cases of poor preservation, these hard structures offer the only means of identification. There is, however, one difficulty with the Krumbach system: it requires so minute an examination that it is only after one has worked at it for some time that he is able to see the distinctive

features with certainty. Fowler's papers, accurate and careful in most details, offer, by far, the best aid to the systematist. The characters used are carefully chosen and tested by an examination of a large series of individuals of each species. Anyone attempting to work on this group will find both the Biscayan and Siboga Reports of this investigator invaluable.

In the genus *Sagitta* I have adopted the synonymy of Fowler ('06), who recognizes eighteen valid species, as shown in the table at the close of this paper. In addition there are several doubtful species: *Sagitta hispida* Conant, *S. tenuis* Conant, *S. maxima* Conant, *S. bedfordi* Doncaster, *S. septata* Doncaster, *S. elegans* Verrill, and *S. arctica* Aurivillus. Descriptions of these species have been so incomplete and drawings so few that, upon the available data, it is impossible to determine their validity. *S. arctica* is very possibly a synonym for *S. elegans* but, until the original specimens can be redescribed, it is best to leave them in the category of doubtful species. Of all the doubtful species Verrill's *S. elegans* appears to be the most valid, but so little is defined in the description that it will not be included in this paper.

Of the eighteen species only a few have, as yet, been found in American waters. *Sagitta hexaptera*, *S. enflata*, and the doubtful species *S. elegans*, *S. tenuis*, *S. maxima*, and *S. hispida* have been recorded from the Atlantic Coast, by Conant ('95, '96), Verrill ('83), and Stevens ('05). The genus *Spadella* is represented by two species, *Spadella draco*, and the doubtful *S. schizoptera* Conant. *Krohnia* is represented by the single species *Krohnia hamata*. The collections of the University of California from the San Diego region of the Pacific Coast, examined by me, contain the following species of *Sagitta*: *Sagitta serratodentata*, *S. zetesios*, *S. enflata*, *S. hexaptera*, *S. bipunctata*, *S. furcata*, *S. neglecta*, *S. decipiens*, and *S. pulchra*. *Spadella* is represented by the single species *Spadella draco*, only one individual having been taken. *Krohnia* has not, as yet, been recorded.

Most of the material, which I have examined, consists of a large number of specimens obtained from the coast of Southern California by the explorations of the San Diego Marine Biological Laboratory, and from the collections made by the "Albatross" in the explorations of the United States Bureau of Fisheries on

the coast of California in 1904. The method has been to separate the material into three assortments, according as the preservation was excellent, fair, or poor. A large series from each assortment was examined in an attempt to isolate the taxonomic characters, and the results from each individual specimen tabulated. Proceeding on this basis I am in a position to utilize, in all essentials, the characters Fowler ('05, '06) has chosen. In the ensuing pages frequent use will be made of his reports.

Methods of Preservation.

Killing has been tried with various combinations of acids, alcohol, and formalin, but, of all reagents, weak formalin gives, by far, the best results. Other reagents cause unequal contractions, swellings, and gross distortions, thereby ruining the material. Fowler ('05) advises killing separately in weak formalin then, after a short time, transferring them, for permanent preservation, into from 5 to 10 per cent. On the whole this method gives excellent results but I have found, in some cases, that considerable curling results. Specimens in this condition are difficult to handle and measurements are rendered more or less inaccurate. The following method will overcome this curling and otherwise give most perfect results: Each *Sagitta* is placed separately on a dry slide or cover-glass and allowed to remain exposed to the air for several seconds. This causes the animal to stick slightly to the slide. Then hold the slide in a vertical position and apply formalin (5 to 10 per cent.) at the upper end, allowing it to wash over the animal which will be killed before it has time to loosen itself from the slide. Another method of adding formalin, drop by drop, to a jar of *Sagitta* has given excellent results, but, if the formalin is added too fast, the results are uneven, owing to variability in the activity of the individuals, some contracting violently to a very weak solution while others are apparently unaffected by it. The three methods herein stated are all good; the second takes the most time but produces the surest results.

Method of Measurement.

Fowler ('05) advises camera drawings as measurements otherwise taken are frequently erroneous, in many cases not tallying

with the drawings at all. For the general features of the body the camera is necessary, but some measurements are so difficult to discern that one is never quite sure he is tracing correctly. I have found it extremely difficult, in such species as *Sagitta enflata*, to see the cephalic limits of the anterior fin, and have never been able to trace the outline with certainty. I find that an ocular micrometer gives more certain results, with such measurements, than the camera.

Characters Used in Classification.

The internal organs of *Sagitta* consist of the digestive, reproductive, and nervous systems. The first includes a simple straight tube leading from the mouth to the anus and offers no definable diagnostic characters; the two lateral diverticula, used by several writers, would seem to be not real diverticula, but rather results of extreme contraction of the head at death. The size and shape of the vesiculæ seminales depend entirely upon the sexual condition of the animal at the time of capture. The extension of the ovaries varies in the different species but, as they are the last organs to develop, one may find an otherwise mature *Sagitta* with ovaries of any length up to the specific maximum. The shape of the ovaries, whether long and slender, or short and thick, possibly has some significance, but as the length depends largely upon the extent of growth, and the width largely upon the maturity of the ova, too much weight cannot be placed upon these characters. The ova might possibly offer excellent characters for diagnosis, but with our present knowledge, one can never be sure whether they are mature or not. The nervous system, if well worked out for every species, might offer excellent characters, but except for two or three species the nervous system has not been investigated. Should the most excellent characters be offered here, their adoption as a means of ready identification would be inconvenient, owing to the technical methods and delicate work required to bring out the points. We are, therefore, compelled to look to the external characters as our only means of accurate identification.

Even among the external features a few characters have been used in the past, which appear to be worthless for specific deter-

mination. The comparative size of the head would seem to be due to the extent to which contraction occurs at death; if the contraction be slight the head appears larger, if great it appears smaller. This also applies to a less extent to the presence or not of a neck but, allowing for the variation thus produced, it may have some taxonomic significance. Again, the presence or absence of color seems to vary with the individual rather than with the species. Fowler ('05) has obtained, in the same haul, individuals of the same species, some a salmon pink, and others without color. The shape of the fins, whether triangular or half elliptical, would be an excellent character were the fins not so frequently damaged. Fowler ('05) has obtained specimens with a triangular fin on one side which had been rubbed into an ellipse on the other. One is often still less certain, from evidence derived from preserved material, whether the tail fin is truncate or rounded in nature.

The structural features then, which are available for diagnosis, consist of the cephalic armature, musculature, lateral fields, corona ciliata, and proportional measurements of various regions of the body. The table, at the close of this paper, includes all valid toxonomic characters, a discussion of which follows.

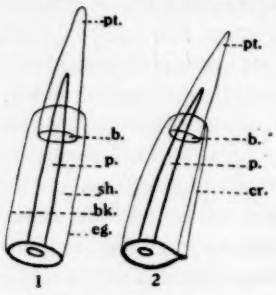
Prior to Krumbach's ('03) paper on "Die Greifhaken der Chaetognathen" practically the only use made, in classification, of the seizing jaws consisted of their enumeration and, even so, the tendency has been to describe the species on a basis of one or two individuals so that not enough latitude has been left for variation within the species. The number of seizing jaws is a very important matter and should be tabulated, together with the number of teeth and length of specimen, for a considerable number of individuals. When the number of seizing jaws is combined with their anatomical characters, as elaborated by Krumbach ('03), they present excellent criteria for identification, and, in many cases, where preservation is poor, practically the only safe criteria. Krumbach ('03) has defined the differences among the various species in the form and curvature of the seizing jaws, the presence and extent of a crest along the shaft, presence and nature of serrations, curvature and shape of the points, extent to which the point is inserted into the shaft, extent

of the pulp into the point, and the pattern of the pulp. Unfortunately all known species were not studied by Krumbach. His method should certainly be extended to the remaining species. His classification is here briefly summarized.

The nine species of Chætognatha studied by Krumbach ('03) were *Sagitta bipunctata*, *S. enflata*, *S. hexaptera*, *S. furcata*, *S. serratodentata*, *S. minima*, *Spadella draco*, *Krohnia hamata*, and *Sagitta magna*, the latter, according to Krumbach ('03), and Fowler ('06), probably being a variety of *S. hexaptera*. These several species Krumbach divides into four groups as follows:

GROUP 1 (Figs. 1 and 2).

Point *pt* with an oval base *b*: strongly needle-shaped. Pulp *p* extends along the central axis of the shaft. Upper third of shaft strongly bent. This group includes two species, *Sagitta bipunctata*, and *Spadella draco*.



Sagitta bipunctata (1).

Point *pt* imbedded one third its height into the shaft *sh*. Shaft with fine longitudinal furrows on the surfaces between the back *bk* and the edge *eg*.

Spadella draco (2).

Flat broad edged crest *cr* on edge of shaft. Point *pt* inserted one fourth to one fifth its height into shaft. Old jaws have serrations on the lower end of the shaft, which dwindle in size as they approach the point, disappearing entirely while still some distance from the point.

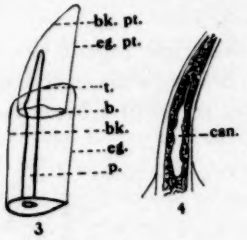
GROUP 2 (Figs. 3, 4 and 5).

Point *pt* with an oval base *b*. Back of point *bk.pt* has greater curvature than the back of the shaft *bk*; edge of point *eg.pt* and edge of shaft *eg* have the same curvature so that the junction of the back and edge of the point at the apex lies toward the edge. Pulp *p* runs slightly nearer the back of the shaft *bk*. Shaft evenly

and slightly curved. This group includes *Sagitta enflata* and *S. furcata.*

Sagitta furcata (3 and 4).

Base of point *b* and top of shaft *t* converge as they approach the back of the shaft *bk.* Cross-section of the shaft is a slender wedge-shape. Pulp *p* is displaced toward the back of the shaft



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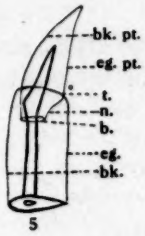

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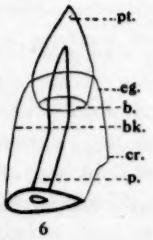
bk. Pulp *p* is concentrated around a canal *can,* which is of a cucumber form and extends to the upper third of the shaft. Old jaws with a small crest.

Sagitta enflata (5).

Base of point *b* and top of shaft *t* converge as they approach the edge of the shaft *eg.* Edge of point *eg.pt* near the base *b* is notched *n.* Cross-section of the shaft more of an oval than in *S. furcata.* Canal irregularly distributed through the pulp.



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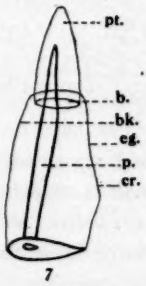

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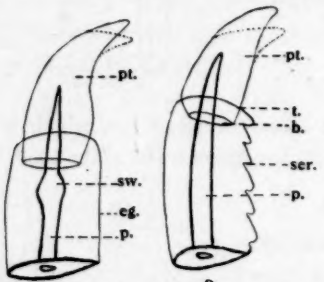
GROUP 3 (Figs. 6 and 7).

Point *pt* with a broad oval base *b.* Pulp *p* slightly toward back of the shaft *bk.* Base of point *b* makes a right angle with the back of the shaft *bk.* Angle between base of point *b* and edge of shaft *eg* is acute. Shaft below point with a short massive crest *cr.* Edge of crest extends proximally on a line with the edge of the point for some distance and then makes an abrupt turn toward the shaft. Shaft evenly and strongly

curved. This group includes *Sagitta hexaptera* (6) and *S. magna* (7). The latter is, as previously mentioned, now regarded as a variety of *S. hexaptera*. The only difference is that the jaws of *S. magna* are finer, more slender, and delicate than in an individual of *S. hexaptera* of the same size. The pulp reaches higher toward the apex of the point than in *S. hexaptera*.

GROUP 4 (Figs. 8, 9 and 10).

Point *pt* sickle-shaped, bent toward the edge of the shaft *eg*, varying in curvature between the two extremes indicated. Base more or less oval. Pulp *p* enters the shaft between the center



8 9 10

and the back focus of the cross-section. Pulp reaches nearly to the knee of the point, but never makes the bend. Shaft only slightly curved. This group includes *Sagitta minima*, *S. serratodentata* and *Krohnia hamata*.

Sagitta minima (8).

Jaws slender and long with slender points only slightly set into the shaft. Cross-section of shaft wedge-shaped. Pulp *p* is swollen *sw* slightly below the point. Tip of point *pt* bent much more than the rest of the jaw. Old jaws have a small delicate crest.

Sagitta serratodentata (9).

Pronounced serrations *ser* on the shaft. The first tooth of the serration is the smallest, extending from the top of the shaft *t* half-way to the basal line of the point *b*. Proceeding proximally the teeth increase in size. Cross-section of the shaft is an

elongated wedge. Old jaws are supplied with a pronounced crest.

Krohnia hamata (10).

Jaws heavy with broad oval cross-section. Pulp *p* only scantily fills the center of the canal *can.* Pulp swollen *sw* as in *S. minima.* Base of point *b* and top of shaft *t* converge toward the edge of the shaft *eg.*

Taken in connection with the seizing jaws the teeth afford an excellent means of identification. Their arrangement into two pairs of rows distinguishes *Spadella* and *Sagitta* from *Krohnia,* which has but one. Within the genus *Sagitta* a glance at the table at the close of this paper will indicate the importance of mere number. It is advisable that the limits of variation for every length of individual be accurately known. Were this accomplished, by combining the number of anterior and posterior teeth with the length of individual, a criterion of identification would result of much value to the systematist.

The form and arrangement of the teeth, if properly recorded, will considerably assist in identification. Some teeth are slender, some broad, so that proportional measurements of width and length are desirable; the length of tooth should also be compared to the length of individual so as to define exactly what is meant by saying that the posterior teeth of *Sagitta hexaptera* are long and slender, while those of *S. ferox* are long and broad. In any group like the Chætognatha, where, at best, identification is difficult, we should endeavor to rid ourselves of characters based upon mere comparisons. All such characters may, at the expense of a little labor, be reduced to exact or proportional measurements. If one merely says that the posterior teeth of *Sagitta siboga* "are long, broader, and with narrower bases than in *bedoti,*" anyone attempting to identify a *S. siboga* would wonder whether the teeth were absolutely broader, broader in proportion to the length of tooth, or broader in proportion to the length of individual.

The inclination of the teeth, whether upright, externally or internally oblique; the proximity of setting, whether close or distant, are of some importance. Fowler ('05, '06) has used

some of these characters but, as yet, they are in a comparative form lacking in that element of exactness and definition so necessary for accurate classification by one unfamiliar with the group.

Another important character is present in the vestibular ridge. The presence of high or low, numerous or few, blunt or acute papillæ are matters that need more attention in the future. The position and height of the papilla should be compared to the position and height of the tooth, and similarly their number should be compared to the number of teeth. Comparison of the extent of the ridge with that of the tooth row proves of some importance; whether it is longer or shorter than the tooth row, and if shorter how many teeth project beyond; whether it terminates abruptly or gradually, in a lateral process or not.

The fins offer good characters if the specimen is well preserved. The presence or absence of a second pair of lateral fins serves to distinguish between *Sagitta* and the other two genera. Relation of the anterior to the posterior fins in length and breadth has been found to be a useful character. In *Sagitta hexaptera*, *S. macrocephala*, *S. neglecta*, *S. bipunctata*, *S. robusta*, *S. serratodentata*, and *S. planctonis* the posterior fin is longer then the anterior fin; in *Sagitta ferox*, *S. pulchra*, *S. zetesios*, *S. whartoni*, and *S. siboga* the posterior fin is shorter; in *Sagitta bedoti*, *S. enflata*, *S. regularis*, *S. furcata*, and *S. decipiens* both fins are the same length. Here again, it is necessary to know how much variation to allow for those in the third group, and to accurately determine this requires measurements of a large series of individuals of each species.

The distance from the anterior to the posterior fin, as measured in per cent. of the total length of the individual, may be made of considerable service. From the table we see that, in *Sagitta bedoti*, this distance measures but 5.4 per cent., the least I have found, except in the case of *Sagitta whartoni* where the fins are confluent, while *S. siboga* measures approximately 15 per cent. The other species are distributed between these extremes. While the measurements are not extensive enough to be of much value, still we can separate the species into two groups; those in which this interval is more than eight per cent. and those in which it is less than eight per cent. In making this separation we still

allow sufficient variation to feel quite safe, except in the case of *Sagitta bipunctata* (7.9 per cent.) and possibly *S. serratodentata* and *S. macrocephala* (7.5 per cent.).

The limits of the anterior fin as tested by the ventral ganglion, and the position of greatest width are of some value to the systematist, in individuals well preserved. In *Sagitta ferox*, *S. neglecta*, *S. pulchra*, *S. whartoni*, *S. serratodentata*, *S. siboga*, *S. zetesios*, and *S. planctonis* the fin extends to the ganglion; in *Sagitta enflata*, *S. hexaptera*, and *S. bipunctata* the fin is remote from the ganglion. The remaining species occupy various points between these two extremes. Until the extent of this variation is better known, for each species, separation cannot be rendered any more definite, by this criterion.

The posterior fin is more instructive. The position of greatest width, as tested by the tail septum; the proportion of posterior fin in front of the septum; the proximity of the fin to the vesiculæ seminales, are all serviceable characters. The table shows the grouping of the species in this matter.

The corona ciliata has been considerably used in the past. Length and width in proportion to the total length, shape and location, how much on head, how much on body, — these form the important features. The great difficulty in using this character lies in the fact that it is very rarely present in preserved material. With living material the corona might have considerable significance, but with formalin material nearly all specimens have apparently lost the structure.

The collarette and lateral fields are of some utility. The collarette or neck fin is an expansion of the ectoderm in the region of the neck and appears as a constant specific character. Some species are always provided with it as *Sagitta siboga*, *S. ferox*, *S. neglecta*, *S. regularis*, *S. pulchra*, *S. robusta*, *S. zetesios*, and *S. decipiens*. It is absent in the remaining species. The lateral fields are those areas between the muscles so that, in general, the presence of large lateral fields is co-existent with weak muscles. Species in this category are very often flabby and transparent in formalin. Formalin acts upon the muscles causing opacity and firmness so that the species with strong longitudinal muscles are readily separated from those with weak mus-

cles. The table indicates the distribution of these characters among the species.

Finally the general shape of the animal is of prime importance. Length, breadth, proportional length of tail, extent or absence of a constriction at the tail septum, are all points to be noted. In some species the body tapers gradually from head to tail, in others a sudden diminution occurs at the tail septum; some are of the same width throughout the entire trunk, others are much wider in the middle of the trunk tapering toward head and tail. Matters of this kind, while extremely useful, are difficult of diagnostic description so that camera drawings present the most accurate means of exhibiting these relations.

KEY TO THE GENERA OF CHÆTOGNATHA.

1. Two pairs of lateral fins. Two pairs of rows of teeth. Only slight epidermal thickening on body ..... *Sagitta.*
2. One pair of lateral fins, partly on body and tail. One pair of rows of teeth. Body longer than tail. No epidermal thickening behind the head..... *Krohnia.*
3. One pair of lateral fins, entirely upon the tail segment. Two pairs of rows of teeth. Prominent thickening of epidermis extending from behind head to tail. *Spadella.*

KEY FOR THE DETERMINATION OF THE SPECIES OF SAGITTA TO BE USED FOR LIVING OR PERFECTLY PRESERVED MATERIAL.

1. Species with collarette..... 12.
1. Species without collarette..... 2.
2. Shaft of seizing jaw serrated..... *Sagitta serratodentata.*
2. Shaft of seizing jaw not serrated..... 3.
3. Irregular transverse septa present on trunk..... *Sagitta minima.*
3. Irregular transverse septa absent on trunk..... 4.
4. Posterior teeth 12 to 32 in number..... 5.
4. Posterior teeth 1 to 12 in number..... 8.
5. At least 50 per cent. of the posterior fin in front of the septum..... 6.
5. Less than 50 per cent. of posterior fin in front of septum..... *Sagitta bedoti.*
6. Tail 28 to 40 per cent. of total length..... *Sagitta macrocephala*
6. Tail 16 to 25 per cent. of total length..... 7.
7. Middle third of body of equal width..... *Sagitta bipunctata.*
7. Body much wider at middle of the length..... *Sagitta enflata.*
8. Length of anterior fin from 44 to 66 per cent. of total length, confluent with the posterior fin. ..... *Sagitta whartoni.*
8. Length of anterior fin less than 40 per cent. of total length, always an interval between anterior and posterior fins..... 9.
9. Anterior fin on a level with ventral ganglion..... *Sagitta furcata.*
9. Anterior fin remote from ventral ganglion..... 10.
10. Posterior teeth less than 8 in number ..... *Sagitta hexaptera.*

10. Posterior teeth more than 8 in number ..... 11.
11. Middle third of body of equal width ..... *Sagitta bipunctata.*
11. Body much wider at middle of length..... *Sagitta enflata.*
12. Irregular transverse septa present on trunk ..... *Sagitta minima.*
12. Irregular transverse septa absent on trunk ..... 13.
13. At least 50 per cent. of posterior fin in front of septum..... 14.
13. Less than 50 per cent. of posterior fin in front of septum..... 18.
14. Posterior fin widest in front of septum..... *Sagitta zetesios.*
14. Posterior fin widest at or behind septum..... 15.
15. Posterior fin shorter than anterior fin..... 16.
15. Posterior fin as long or longer than anterior fin..... 17.
16. Posterior teeth 9 to 15 in number ..... *Sagitta pulchra.*
16. Posterior teeth 15 to 23 in number ..... *Sagitta siboga.*
17. Anterior fin reaching to middle of ventral ganglion ..... *Sagitta planctonis.*
17. Anterior fin not reaching to ventral ganglion..... *Sagitta decipiens.*
18. Posterior fin shorter than anterior fin..... *Sagitta ferox.*
18. Posterior fin as long or longer than anterior fin..... 19.
19. Corona ciliata extends anteriorly beyond eyes ..... *Sagitta robusta.*
19. Corona ciliata not extending to eyes..... 20.
20. Middle third of body of equal width..... *Sagitta neglecta.*
20. Body of equal width from the ventral ganglion to the seminal vesicles. *Sagitta regularis.*

If (19) the corona ciliata is absent the following criteria may be used :

19. Middle third of body equally wide..... *Sagitta neglecta.*
19. Body equally wide from ventral ganglion to the seminal vesicles.. *Sagitta regularis.*
19. Body equally wide from just behind the neck to immediately in front of the tail septum..... *Sagitta robusta.*

KEY FOR DETERMINATION OF THE SPECIES OF SAGITTA TO BE USED FOR POORLY PRESERVED MATERIAL.

1. Species with collarette..... 12.
1. Species without collarette..... 2.
2. Shaft of seizing jaws serrated..... *Sagitta serratodentata.*
2. Shaft of seizing jaws not serrated..... 3.
3. Irregular transverse septa present on trunk..... *Sagitta minima.*
3. Irregular transverse septa absent..... 4.
4. Length of anterior fin from 44 to 66 per cent. of the total length, anterior fin and posterior fins confluent..... *Sagitta whartoni.*
4. Length of anterior fins never as much as 40 per cent. of total length, always an interval between anterior and posterior fin..... 5.
5. Tail 16 to 27 per cent. of the total length..... 6.
5. Tail 28 to 35 per cent. of total length..... 7.
6. Posterior teeth less than 8 in number..... 8.
6. Posterior teeth more than 8 in number..... 9.
7. Jaws more than 8 in number..... *Sagitta macrocephala.*
7. Jaws less than 8 in number..... *Sagitta bedoti.*
8. Anterior teeth 1 to 4 in number..... *Sagitta hexaptera.*

8. Anterior teeth more than 4 in number.....*Sagitta furcata.*
9. Body firm and opaque; tail slender.....*Sagitta bipunctata.*
9. Body transparent in formalin.....10.
10. Body flabby and easily compressed.....*Sagitta furcata.*
10. Body firm.....11.
11. Neck very marked.....*Sagitta enflata.*
11. Neck almost missing.....*Sagitta bedoti.*
12. Irregular transverse septa present on trunk.....*Sagitta minima.*
12. Irregular transverse septa absent.....13.
13. Neck well marked.....14.
13. Without neck.....18.
14. Jaws more than 8 in number.....15.
14. Jaws less than 8 in number.....16.
15. Collarette extends to anterior fin.....*Sagitta zetesios.*
15. Collarette never extends over half the distance to the anterior fins. *Sagitta planctonis.*
16. Body thickest in the middle third.....*Sagitta siboga.*
16. Body of uniform thickness from ventral ganglion to tail.....17.
17. Collarette extends to anterior fins.....*Sagitta ferox.*
17. Collarette never extends over half the distance to the anterior fins. *Sagitta robusta.*
18. Posterior teeth less than 7 in number.....*Sagitta regularis.*
18. Posterior teeth more than 7 in number.....19.
19. Body opaque.....20.
19. Body transparent.....21.
20. Body equally thick from just behind neck to the tail septum.....*Sagitta robusta.*
20. Body thicker in the middle third, gradually tapering toward head and tail. *Sagitta neglecta.*
21. Body width about 10 per cent. of total length, tail less than 27 per cent. of total length.....*Sagitta pulchra.*
21. Body width about 5 per cent. of total length, tail more than 27 per cent. of total length.....*Sagitta decipiens.*

Key for the Determination of the Species of Krohnia.

1. Lateral fin extends from the region of the ventral ganglion to somewhat behind the tail septum; never reaches to seminal vesicles; more than 60 per cent. in front of tail septum.....*Krohnia hamata.*
1. Cephalic limit of lateral fin never more than half the distance from the tail septum to the ventral ganglion; extends caudally to the seminal vesicles; more than 40 per cant. of fin behind septum.....2.
2. Body width about 4 per cent. of total length. Tail 30 to 40 per cent. of total length. Seizing jaws evenly curved, 6 to 9 in number. Teeth 9 to 13. *Krohnia subtilis.*
2. Body width about 5 per cent. of total length. Tail 25 to 41 per cent. of total length (usually not over 33 per cent.). Teeth 11 to 15. Seizing jaws 6 to 9, with the convex edge made up of two curves, the junction of which makes an obtuse angle at a point about one fourth the length of the jaw proximad of the tip. *Krohnia pacifica.*

KEY FOR THE DETERMINATION OF THE SPECIES OF SPADELLA.

1. Average width of collarette nearly half that of the body, widest slightly anterior to the tail septum. Length of fin always less than 5 times its width. Anterior teeth 7 to 10. Posterior teeth 11 to 16.....*Spadella draco.*
2. Average width of collarette much less than half that of the body; widest slightly posterior to the head. Length of fin always more than 5 times its width. Anterior teeth 3 to 5. Posterior teeth 3 to 4.....*Spadella cephaloptera.*

DESCRIPTION OF TABLE.

The data for this table have been gathered from various sources, but mostly from the investigations of Fowler ('05, '06) and Krumbach ('03). In proportional measurements, number of seizing jaws, and number of teeth, the extremes of variation have been indicated wherever possible.

In the case of *Sagitta whartoni* the data have been obtained from Fowler's "Contributions to our Knowledge of the Plankton of the Faroe Channel," 1896. In this instance a considerable discrepancy, due in all probability to a typographical error, is found in comparing the drawing with the descriptive measurements. From Fowler's description the width of the anterior fin varies from 10 to 15 per cent. of the total length; from his drawing it measures but 2.8 per cent. of the total length. Similarly, in the width of the posterior fin, his measurements show variation from 13 to 21 per cent. of the total length, while his drawing shows it but 5.7 per cent. In no other species has the width of the anterior fin been over 6 per cent. of the total length, nor the width of the posterior fin over 7 per cent. For this reason I have utilized the fin measurements as taken from the drawing rather than from the description of *Sagitta whartoni.*

I desire to express my obligations to Dr. C. A. Kofoid, through whose direction, advice, and criticism I have received invaluable aid.

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Explanation of Plate.

Fig. 1. *Sagitta hexaptera* × 2. *ant.t.*, anterior teeth; *post.t.*, posterior teeth; *s.j.*, seizing jaws; *al.can.*, alimentary canal; *vent.gang.*, ventral ganglion; *ant.f.*, anterior fin; *post.f.*, posterior fin; *t.s.*, tail septum; *t.*, tail; *t.f.*, tail fin.

Fig. 2. *Sagitta neglecta* × 2. *col.*, collarette; *vent.gang.*, ventral ganglion; *ant.f.*, anterior fin; *post.f.*, posterior fin; *t.s.*, tail septum; *t.*, tail; *sem.ves.*, seminal vesicles; *t.f.*, tail fin.

Fig. 3. *Sagitta robusta* × 13. (After Fowler.) *e.*, eye; *cor.cil.*, corona ciliata; *col.*, collarette; *vent.gang.*, ventral ganglion.

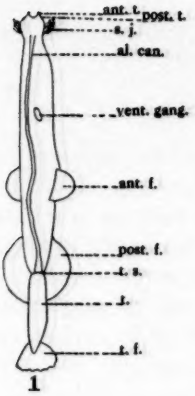
Fig. 4. *Sagitta regularis* × 22. (After Fowler.) *e.*, eye; *cor.cil.*, corona ciliata; *col.*, collarette; *vent.gang.*, ventral ganglion.

Fig. 5. × 346. Vestibular ridge and posterior teeth of *Sagitta neglecta*.

Fig. 6. Typical seizing jaw. (After Krumbach.) *pt.*, point; *ap.p.*, apical pulp; *out.mem.*, outer membrane; *cr.*, crest; *bk.*, back of shaft; *eg.*, edge of shaft; *p.*, pulp; *p.can.*, pulp canal; *vent.col.*, ventral column; *dor.col.*, dorsal column; *mus.at.*, muscle attachment; *b.p.*, basal pulp.

Fig. 7. *Spadella draco* × 8. *col.*, collarette; *vent.gang.*, ventral ganglion; *t.s.*, tail septum; *lat.f.*, lateral fin; *t.*, tail; *t.f.*, tail fin.

Fig. 8. *Krohnia hamata* × 3. (After Fowler.) *vent.gang.*, ventral ganglion; *lat.f.*, lateral fin; *t.s.*, tail septum; *t.*, tail; *t.f.*, tail fin.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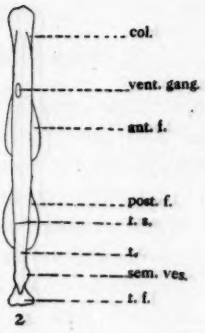
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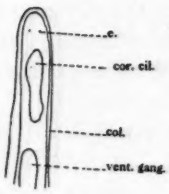
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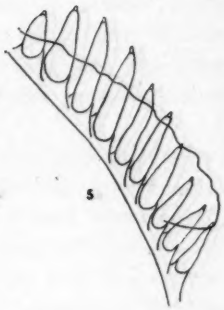

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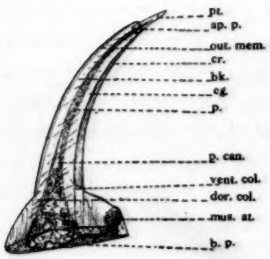
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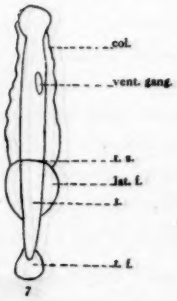
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8

EFFECTS OF ALCOHOL ON THE LIFE CYCLE OF INFUSORIA.

LORANDE LOSS WOODRUFF.

So many investigations have been undertaken to determine the effects of alcohol on the higher animals and with such varied results, that it is of interest to determine its effect upon the lowest and most generalized animal organisms, the Protozoa. As single-celled animals these forms cannot fail to give results of importance from the point of view of general cell-physiology, and lead to a clearer analysis of the primary effects of alcohol on metabolism. The Protozoa are particularly well fitted for cellular-physiological study, not only because as single free-living cells they lend themselves readily to experimental methods, but also because no one function predominates at the expense of the rest, and results obtained may reasonably be supposed to be due to the effects of the stimulus in question on the general metabolism of the cell.

Hunt[1] recently made some interesting experiments on the effect of small doses of alcohol on mice and guinea-pigs, and found that animals to which alcohol has been administered for some time acquire increased susceptibility to a definite poison (acetonitrile), and reached the conclusion that this increase in susceptibility is not due to a general "lowering of resistance," but is associated with a distinctly increased power of the body to break up the molecule of acetonitrile.

Calkins and Lieb[2] carried on some experiments with alcohol on *Paramecium* and found that ". . . alcohol has no effect when taken in too weak doses, and too powerful an effect when taken in over strong doses." ". . . when a medium dose is given (for example 3 parts of 1/1,000 alcohol to 2 of hay, or 1 part of

[1] Reid Hunt, "Studies on Experimental Alcoholism," Bull. No. 33, Hyg. Lab., U. S. Pub. Health and Mar.-Hosp. Serv., Washington, 1907.

[2] Gary N. Calkins and C. C. Lieb, "Studies on the Life-History of Protozoa,—II. The Effects of Stimuli on the Life-Cycle of *Paramecium caudatum*," *Archiv für Protistenkunde*, 1902.

1/500 alcohol to 4 parts of hay) the effect is a continued stimulus which sustains the high rate of division even during periods of depression of the control series." "There is no doubt that for a time at least, alcohol will prevent death during periods of depression. . . ." ". . . there is evidence that . . . the general vitality would decrease under the constant stimulus as it does under treatment with hay infusion alone, although much more slowly." "Notwithstanding the more rapid living, the general vitality does not seem to be affected badly by the alcohol." It was chiefly to determine the latter point that this investigation was begun, and I shall outline the progress of the work to the present time.

II. Methods.

I chose *Paramecium aurelia*[1] for the main line of experiments chiefly because considerable work has already been performed on this organism; and because it is one of the more generalized of the ciliates; and lastly because its cosmopolitan distribution renders it a convenient form to be studied in all laboratories. It seems to me to be more desirable, in the present state of our knowledge, to learn one form thoroughly, if that is possible, rather than to distribute our energies over a broader field. As a subsidiary line, for comparison and as a check on the *Paramecium* cultures, I employed a culture of *Stylonychia mytilus*.

The general method of carrying the cultures is identical with that which has been described in detail in an earlier paper,[2] so that a brief outline at this time will suffice.

A "wild" individual was captured and placed on a depression slide in five drops of hay infusion. This infusion was made by putting about three grams of hay or grass in 200 c.c. of tap water and then raising the temperature to the boiling point. This infusion was generally used as soon as it had again attained the room temperature. It was made fresh daily as a rule. Sufficient bacteria developed to provide ample nourishment for the infusoria, and since all precautions were taken in selection of the hay, etc.,

[1] The specific name *aurelia*, instead of *caudatum*, is adopted in accordance with the data advanced by Calkins, "*Paramecium aurelia* and *Paramecium caudatum*," Biol. Stud. Pupils of W. T. Sedgwick, Chicago, 1906.

[2] Lorande Loss Woodruff, "An Experimental Study on the Life-History of Hypotrichous Infusoria," *Journal of Experimental Zoölogy*, II., 4, 1905.

it is believed that a satisfactory culture medium for comparative work was obtained. When the isolated protozoön had divided twice, producing four individuals, each was isolated on a separate depression slide and thus were started the four lines of which each culture consisted. Thus, for example, *Paramecium*, culture I, comprised four lines, *I–a*, *I–b*, *I–c* and *I–d*. These lines were thenceforth kept distinct unless one became extinct through the isolation of a weak individual or through accident, in which case the line was started again from one of the three surviving sister lines of the same culture.

The rate of division was recorded daily for each of the four lines, and at the time of record an individual from each line was isolated on a clean depression slide in five drops of hay infusion. In computing the rate of division of the culture as a whole, with which we are alone concerned, the four lines (*a*, *b*, *c*, *d*) of the culture were averaged together and this result was again averaged for five-day periods. By this method it is believed that a just conception of the rate of division of the culture was obtained, as the average of the four lines largely obliterated the fluctuations in the division rate of any one line, which may not have been of much significance, or which may have been merely due to the isolation of a weak individual. One who has carried on cultures of protozoa for considerable periods cannot fail to recognize the fact that, as is to be expected, individuals vary greatly in their general vitality, etc., and it is necessary to isolate representative individuals. It is here that the personal equation of the experimenter comes into view, and therefore it is desirable that the same person should make the daily isolations.

The culture slides were arranged in small moist chambers to prevent evaporation. As in previous experiments of this nature, the minimum and maximum temperature of the room in the vicinity of the cultures was recorded daily, as indicated by a registering thermometer. By averaging the minimum and maximum points of each day for five-day periods the results obtained are quite satisfactory for comparative work. In the experiments under consideration, as in those of previous investigators in this field, the rate of division was taken as the indication of the physiological condition of the organisms; it being generally accepted

that this is the most accurate indication of the general metabolic condition of the protoplasm of organisms which is available.

The work was started at the Thompson Biological Laboratory of Williams College in the spring of 1907; was continued at the Woods Holl Marine Biological Laboratory during the summer, and is at present being carried on at the Sheffield Biological Laboratory of Yale University.

III. Details of Control Cultures.

(*a*) *Paramecium aurelia.*

The "wild" individual with which this culture (*Paramecium* I) was started was isolated from an infusion in the Williams laboratory on May 1, 1907, and has been kept continually under observation since that time, and is at present (January 25, 1908) in the 332d generation. The average rate of division of the four lines (*I–a*, *I–b*, *I–c*, *I–d*), again averaged for each five-day period during the life of the culture up to present time, is plotted by the familiar block method. By glancing at Figs. 1 and 2 (continuous line) it will be seen that the culture started off with a division rate of just two divisions per day, and at the present time, period 54, is averaging one and three quarters divisions per day.

I shall not attempt to analyze the division rate of the culture in relation to the life cycle of *Paramecium* at the present time, as in the experiments under consideration it is of interest solely as the "control."

(*b*) *Stylonychia mytilus.*

This culture (*Stylonychia* I) was started with a "wild" individual found in an infusion in the Yale laboratory on October 23, 1907, since which time it has been under daily observation and has been subjected to the same method and treatment as the *Paramecium* culture. It is at present (January 25, 1908) in the 165th generation. The rate of division is plotted in Fig. 3.

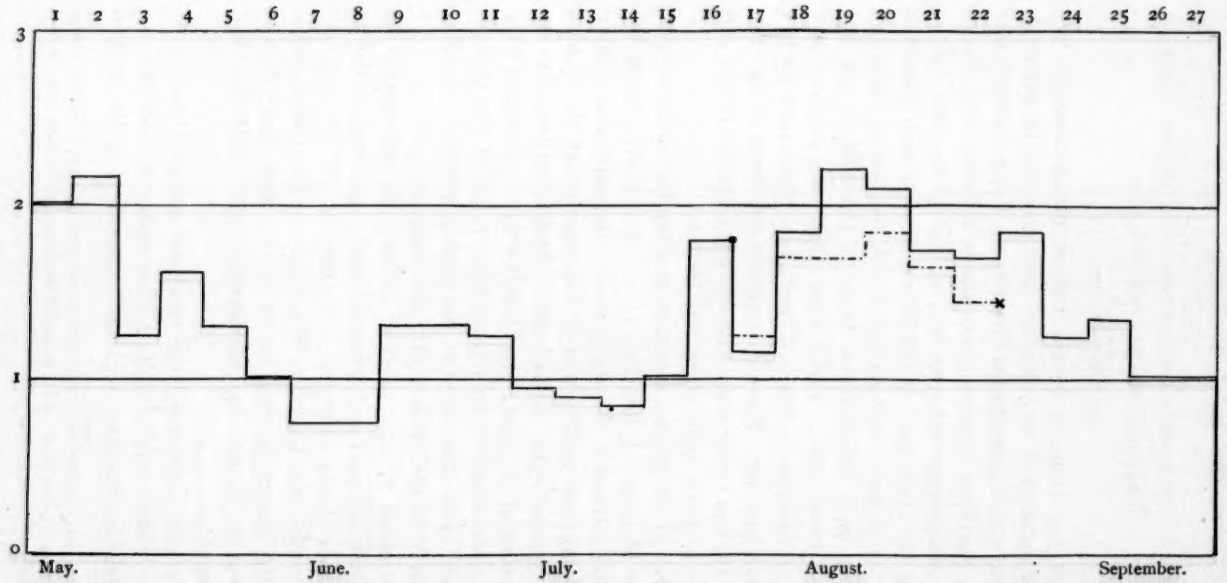

FIG. 1. Life-history of *Paramecium aurelia* (periods 1 through 27). Culture I (hay infusion) = continuous line; culture I^1 (subjected to 1/2,500 alcohol) = · - · - · line. ● = point of isolation of culture I^1 from culture I. × = point at which culture I^1 was discontinued. The figures above the diagram represent the five-day periods for which the rate of division is averaged. The figures at the left represent the average number of divisions per day of the four lines.

IV. General Effect of Alcohol on the Division Rate of Paramecium and Stylonychia.

(*a*) *Paramecium.*

On July 19, 1907, a second culture of *Paramecium* was started by isolation of an individual from each line of culture I. This second culture, designated *Paramecium* I^1, was carried under identically the same conditions as culture I, being subjected to the same temperature changes, etc., and given the same culture medium at the same time, except that instead of each individual receiving five drops of hay infusion (as in the case of culture I, the control), each received four drops of hay infusion and one drop of 1/500 alcohol. There were, then, two cultures each consisting of four lines which had been under observation for two and one half months. The only apparent difference in the conditions of the two cultures was that one was subjected to one part of alcohol to 2,500 parts of culture medium.[1]

The effect of the alcohol was seen in a slightly increased rate of division of culture I^1 (see Fig. 1, ·---· line) above that of the control during the first five-day period. But after that, during the remaining twenty-five days of the experiment, the alcohol caused a decrease in the division rate. This culture was discontinued on August 19, 1907. In Table I is given the actual daily record of generations of the control culture and of the alcoholized culture, as an illustration of the general method of keeping the records of all the cultures throughout the work.

On November 6, 1907, another culture (I^2) was started by isolation from culture I and given the same treatment as that to which culture I^1 was subjected. A glance at Fig. 2 (·---· line) shows, however, that the effect of alcohol was this time a stimulation of the rate of division, for during the first thirty days of the experiment the rate was considerably more rapid than that of the control.

There is then, comparing the results of culture I^1 and those of the first thirty days of culture I^2, a clear-cut example of a stimulus (alcohol) causing a general retardation of the division

[1] Various amounts of alcohol were tried and the strength here employed was chosen as the one giving the best result. All strengths of alcohol which were tried at this period produced the same general effect.

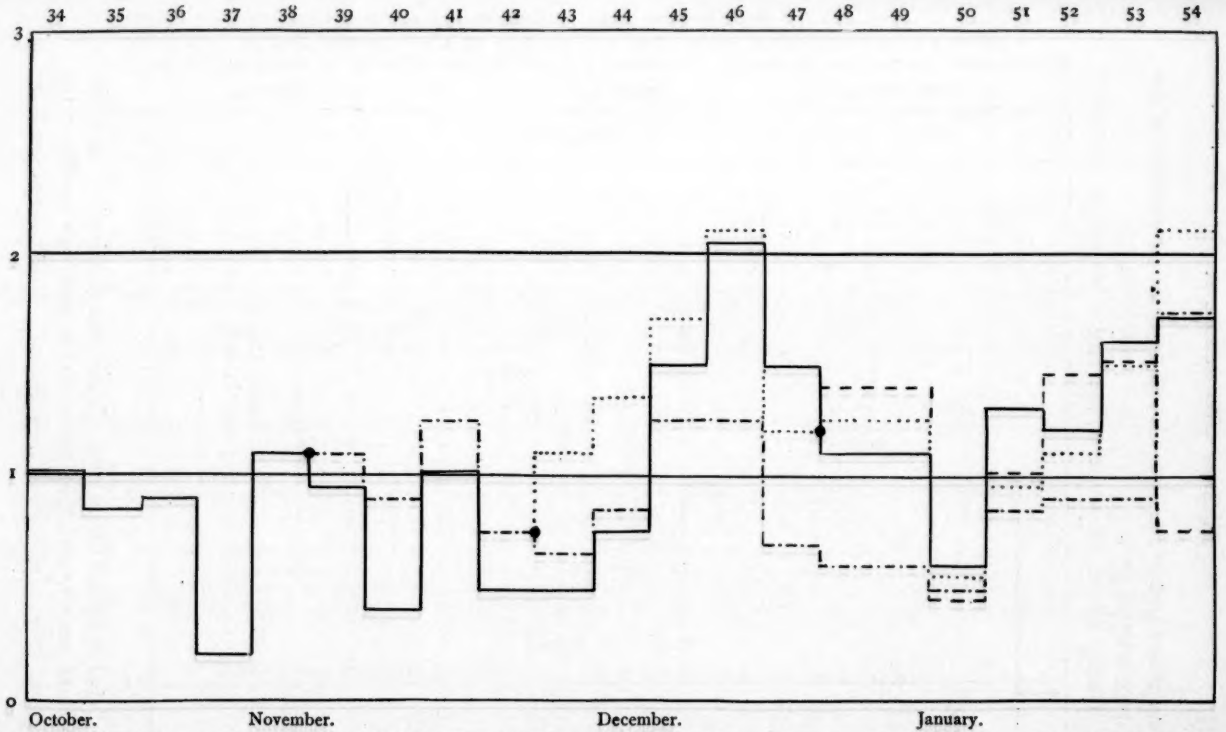

FIG. 2. Life-history of *Paramecium aurelia* (periods 34 through 54). Culture I (hay infusion) = continuous line; culture I^2 subjected to 1/2,500 alcohol = · - · - · - line; culture I^{2a} subjected to 2/2,500 alcohol = · · · · line; culture $I^{2a'}$ subjected to 4/2,500 alcohol = - - - - - line. ● = points of isolation of the respective alcoholized cultures. Other details as in Fig. 1.

rate at one part of the life cycle and a general acceleration of the rate at another part.

During period 45, however, the rate of division of culture I^2

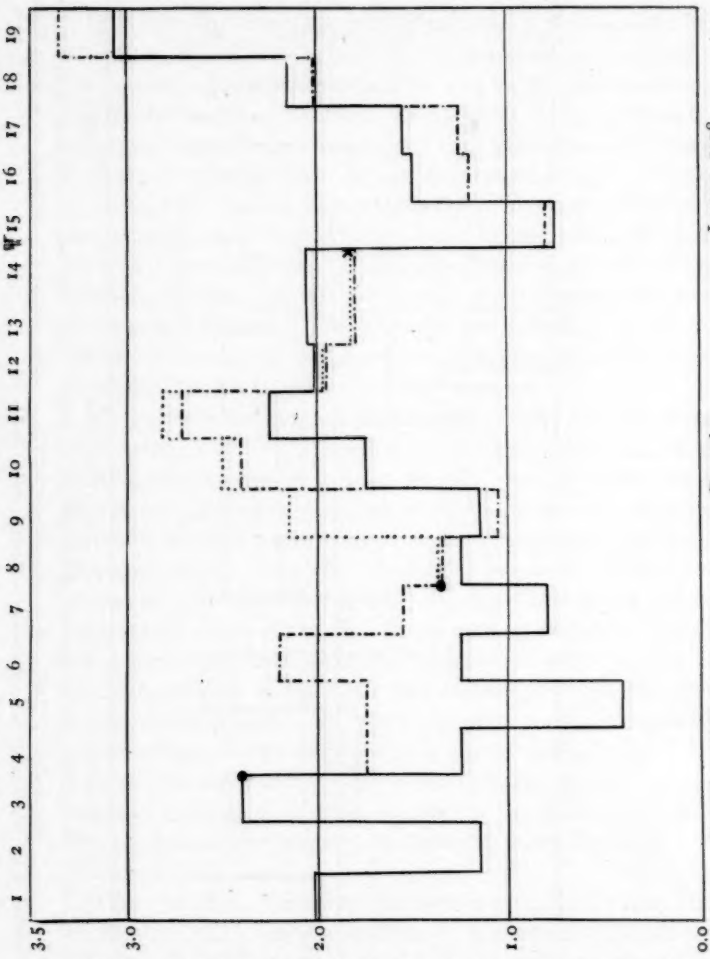

FIG. 3. Life-history of *Stylonychia mytilus* (periods 1 through 19). Culture I (hay infusion, control) = continuous line; culture I^1 subjected to 1/2,500 alcohol = - - - - line; culture I^{1a} subjected to 2/2,500 alcohol = · · · · line; × = point at which culture I^{1a} was discontinued. Other details as in Fig. 1.

fell below that of the control (I) and has remained so up to the present period when it increased to the same rate as that of the control (Fig. 2, periods 45 through 54). It was suspected, how-

PARAMECIUM I.

Date.	July.													August.																
	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Temperature Degrees F.	71/78	71/79	70/77	72/80	71/78	67/79	66/73	72/75	66/76	68/74	69/77	69/76	70/77	69/77	72/79	73/78	66/78	65/68	69/72	69/74	70/78	69/75	66/77	71/76	72/76	71/77	67/76	65/75	68/72	66/71	70/75
Line *a* generations	100	101	101	103	103	b 108	107	110	111	113	115	117	120	121	124	126	128	130	132	134	137	138	141	143	145	147	148	150	151	153	155
" *b* "	99	100	101	102	104	106	107	110	111	113	115	118	120	122	124	126	129	130	133	135	138	139	142	144	146	148	149	151	152	154	156
" *c* "	97	98	98	100	100	102	104	107	108	110	112	115	117	119	121	123	126	127	127	134	138	139	142	144	146	147	149	152	153	155	156
" *d* "	100	101	101	103	105	107	108	110	111	114	116	119	121	123	125	127	129	131	133	135	138	138	140	142	144	146	147	150	151	153	155

PARAMECIUM I[1].

Date.	July.													August.																
	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Temperature Degrees F.	71/78	71/79	70/77	72/80	71/78	67/79	66/73	72/75	66/76	68/74	69/77	69/76	70/77	69/77	72/79	73/78	66/78	65/68	69/72	69/74	70/78	69/75	66/77	71/76	71/76	71/77	67/76	65/75	66/72	66/71	70/75
Line *a* from *I–a.*	100	101	101	104	104	106	108	110	111	113	115	117	119	120	122	123	125	127	129	131	133	134	135	138	139	141	143	146	147	149	151
" *b* " *I–b.*	99	100	101	103	104	105	107	107	108	110	112	113	115	116	118	120	122	123	125	127	130	131	133	135	137	139	140	143	144	146	147
" *c* " *I–c.*	97	98	99	100	101	103	105	107	108	111	113	115	117	118	120	122	124	124	125	127	130	131	134	136	138	140	141	144	145	147	149
" *d* " *I–d.*	100	101	102	104	105	107	108	110	111	113	115	117	119	120	122	124	126	127	129	130	133	133	135	137	139	139	140	140	140	141	141

TABLE I.

Daily Record of Paramecium Cultures I and I[1] from July 19, 1907, to August 18, 1907.

ever, that the accelerating effect of a given strength of alcohol would not be continuous, so at period 43 another culture was started line by line from culture I^2 in identically the same way except that the amount of alcohol administered was doubled, each individual receiving one drop of 2/500 alcohol and four drops of hay infusion. The rate of division of this series, designated culture I^{2a}, at once greatly increased (see Fig. 2, · · · · · line) and kept considerably above the control during the first twenty days of the experiment. During the succeeding periods it fluctuated above and below the control and at the present time is dividing considerably faster than the control culture (Fig. 2 · · · · line).

During the early part of culture I^{2a}, there was again reason to believe that the increase in the division rate would not be permanent, so still another culture ($I^{2a'}$) was isolated from culture I^{2a} (during period 48), and was treated with double the amount of alcohol to which the parent culture was subjected, that is, with one drop of 4/500 alcohol and four drops of hay infusion. The result was still again an initial increase in the rate of division, though of shorter duration, followed by fluctuations above and below the control series (Fig. 2, - - - - line).

In view of the fact that culture I^1 showed a practically uniform depression of the division rate when subjected to alcohol, it was thought that possibly the depression effect which secondarily appeared in cultures I^2, I^{2a}, and $I^{2a'}$, might be due to the fact that the culture as a whole again had attained a period in the life cycle when alcohol had a depressing effect on cell division and, therefore, that the falling off of the rate of division of the organisms, after being a certain length of time subjected to the alcohol, was not due to the animals becoming accustomed to the alcohol. To test this point another culture (I^3) was isolated from the control (I) and carried for three periods of five days each on one drop of 1/500 alcohol and four drops of hay infusion. During this time the division rate of culture I^3 was consistently accelerated and as greatly as that of culture $I^{2a'}$ which was receiving four times as much alcohol, thus showing that the results obtained in the alcohol experiments were due to "acclimatization," and not to the fact that the organisms were in a period of the cycle characterized by a changed susceptibility to alcohol.

(*b*) *Stylonychia.*

On November 6, 1907, a second culture of *Stylonychia* (culture I^1) was started line by line from culture I in exactly the same manner as *Paramecium* I^1 was started from *Paramecium* I. The treatment which followed was identically the same as that already described for the *Paramecium* experiments — culture I^1 being subjected to one part of alcohol to 2,500 parts of culture medium. The effect of this treatment is shown in Fig. 3, beginning at period 4. It will be noted that the division rate of the alcohol treated series (· - · - · line) was very much more rapid than that of the control (continuous line) during the first five five-day periods of the experiment. It fell slightly below the control during the next period, but during the following two periods of the experiment it was again far more rapid than the control. Thus we find in the case of *Stylonychia* that the treatment with alcohol of the strength employed produced, as in the *Paramecium* (culture I^2) experiment, stimulation for about the first month of the work. The plotted curve shows also that from this point on the rate of division of this series fluctuated above and below the control and at the present period it is again exceeding that of the control culture.

On November 26, 1907, culture I^{1a} was started from culture I, and was thenceforth treated with double the amount of alcohol (one drop of 2/500 alcohol and four drops of hay infusion) to which the parent culture was subjected. The result, again similar to that of the corresponding *Paramecium* culture, shows an increased division rate for several five-day periods of the culture subjected to the increased amount of alcohol. (Cf. Fig. 3, period 8 through 14, ····· line.) Again the stimulating effect was not continuous, but instead, as in the previously described experiments, the division rate of the alcohol-treated line finally fell below that of the control and remained below until the experiment was discontinued on December 31, 1907; though a new culture isolated from this culture and stimulated with double the amount of alcohol showed an increased division rate at first, and later decreased division rate as compared with that of the control culture.

V. Does Alcohol Cause a General Lowering of Resistance to Changes in the Environment?

Experiments to determine if treatment with alcohol will cause a general "lowering of resistance" to inimical changes in the environment are in progress, and the experiments described in this paper outline the method which is being employed in such a study.

For this work certain of the previously described cultures are being used, viz.,

Paramecium.	*Stylonychia.*
I = hay infusion.	I = hay infusion.
I^{2} = hay infusion + 1/2,500 alcohol.	I^{1} = hay infusion + 1/2,500 alcohol.
I^{2a} = hay infusion + 2/2,500 alcohol.	I^{2} = hay infusion + 2/2,500 alcohol.
$I^{2a'}$ = hay infusion + 4/2,500 alcohol.	

On December 21, 1907, an individual was isolated from each line of the first three *Paramecium* cultures and from each line of the three *Stylonychia* cultures, and treated in identically the same way as the culture from which they were taken except that each was subjected to one part of copper sulphate in 1,250,000 parts of culture medium.[1] For example, the culture $I\frac{2}{c}$ isolated from *Paramecium* I^{2} received daily three drops of hay infusion plus one drop of 1/500 alcohol plus one drop of 1/250,000 copper sulphate, *i. e.*, it received one drop of 1/250,000 copper sulphate in place of one of the drops of hay infusion received by culture I^{2}.

The six cultures isolated were then as follows:

Paramecium.	*Stylonychia.*
I_c = hay infusion + 1/1,250,000 $CuSO_4$.	I_c = hay infusion + 1/1,250,000 $CuSO_4$.
$I\frac{2}{c}$ = hay infusion + 1/2,500 alcohol + 1/1,250,000 $CuSO_4$.	$I\frac{1}{c}$ = hay infusion + 1/2,500 alcohol + 1/1,250,000 $CuSO_4$.
$I\frac{2a}{c}$ = hay infusion + 2/2,500 alcohol + 1/1,250,000 $CuSO_4$.	$I\frac{2}{c}$ = hay infusion + 2/2,500 alcohol + 1/1,250,000 $CuSO_4$.

The results of these experiments are plotted in Figs. 4–7. These curves show that, in the experiments on *Paramecium*, the alcohol-treated cultures (Figs. 5, 6) died out under the administration of copper sulphate during the fifth period of experimentation, that

[1] Various solutions of copper sulphate were tried and the one employed was selected because it appeared to be the maximum strength which all the cultures could withstand.

is, after being subjected to copper sulphate for twenty-three days, whereas the culture which had never been subjected to alcohol survived under the copper sulphate treatment until discontinued (Fig. 4). A closer analysis of the curves shows that during the earlier periods of the experiment the division rate of the organisms treated with 1/2,500 alcohol was somewhat less affected by the copper sulphate treatment than the division rate of the non-

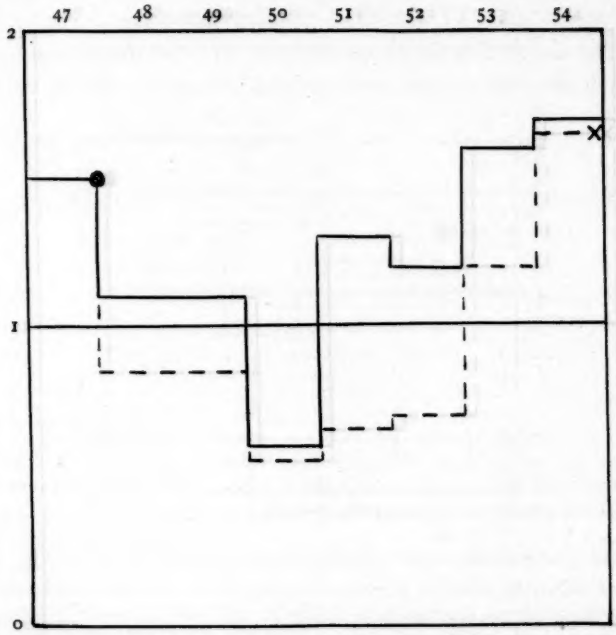

FIG. 4. *Paramecium.* Culture I (hay infusion, control) periods 47 through 54 = continuous line; culture I_e subjected to $CuSO_4$ = - - - - - line. × = point at which culture I_e was discontinued. Other details as in Fig. 1.

alcoholized line, but this was merely temporary. The lines subjected to the greater amount of alcohol (Fig. 6), which were averaging a higher rate of division than the lines treated with the less amount (Fig. 5), were more susceptible to copper sulphate from the beginning than either the non-alcoholized line (Fig. 4) or the line on the less amount of alcohol, and finally died out.

The copper sulphate experiments on the *Stylonychia* culture were carried on for only ten days, but increased susceptibly to copper sulphate is shown by the alcoholized lines (cf. Fig. 7).

The division rate of the three *Paramecium* cultures and the three *Stylonychia* cultures subjected to copper sulphate shows distincly that the organisms which have been treated with alcohol are less resistant to copper sulphate.

VI. General Considerations.

The aim of this paper has been to set forth merely the facts which the experiments have revealed. Several possible causes

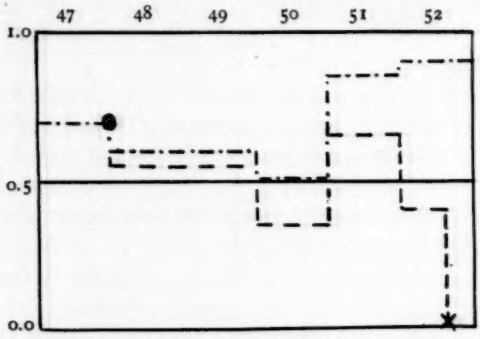

Fig. 5. *Paramecium.* Culture I^2 (hay infusion + alcohol) = ····· line; culture I^2_c (hay infusion + alcohol + $CuSO_4$) = - - - - - line. × = point at which the culture subjected to copper sulphate died out.

of the phenomena observed may be suggested, but it will be of more value to reserve a general discussion of these until more data from experiments are at hand.

Calkins and Lieb, as already mentioned, found that alcohol in medium doses, *e. g.*, one part of alcohol in 2,500 parts of culture medium, acted as a continued stimulus to the division rate of *Paramecium.* The results of my experiments obtained up to the present time fail to show such a marked uniformity of effect from alcohol treatment, as a depression of the rate of division, followed by fluctuations above and below the control, was the result in *Paramecium* I^2 and in all the succeeding cultures of both species, after an initial stimulation of the division rate of a longer

or shorter duration. Since these experiments were conducted with the same general method as that employed by these authors, except that I have carried four lines instead of one line of each alcohol culture and therefore have the average rate of division, the cause of this variation in the results is not apparent. It seems to be clear, however, that alcohol in optimum amounts does usually cause an increase in the rate of division for a certain length of time, and then a falling off of the rate. Whether the

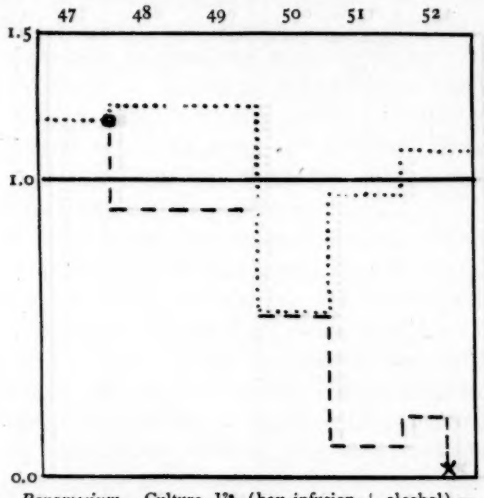

FIG. 6. *Paramecium.* Culture I^{2a} (hay infusion + alcohol) = ····· line; culture $I^{\frac{2a}{c}}$ (hay infusion + alcohol + $CuSO_4$) = - - - - - line. × = point at which the culture subjected to copper sulphate died out.

continued stimulation with alcohol will cause a prolongation of the cycle beyond the normal one of the non-alcoholized lines, as found by Calkins and Lieb, remains to be seen.

It is generally accepted at the present time that alcohol has a tendency to prevent the oxidation of other material in the body by its own oxidation — thus alcohol is a "food" rather than a "drug." Without attempting to consider in detail the question of alcohol as a "food" in relation to the metabolism of the protozoa, about which too little is known, I believe that the effect which alcohol exerts on the division rate of infusoria is not to be

interpreted as an increase in the available food supply of the organisms or as a substitute therefor. Any increase in the number of bacteria in the alcohol cultures due to an effect of alcohol on the reproduction of the bacteria can, I believe, be disregarded

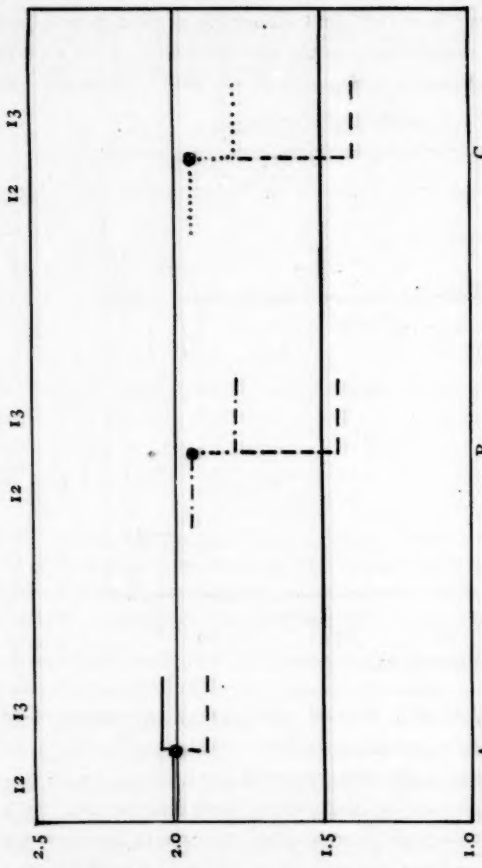

FIG. 7. *Stylonychia*. A. Culture I (hay infusion) = continuous line; culture I_0 subjected to $CuSO_4$ = - - - - line.
B. Culture I^1 (hay infusion + alcohol) = ... line; culture I^1_0 (hay infusion + alcohol + $CuSO_4$) = - - - -line.
C. Culture I^{1a} (hay infusion + alcohol) = ... line; culture I^{1a}_0 (hay infusion + alcohol + $CuSO_4$) = - - - - line.

because the control cultures show that bacteria develop in the freshly made hay infusion far more rapidly than they are consumed by the animals under the conditions of the experiment. If it be assumed that alcohol serves in a limited sense as a food

in that it is more easily oxidized than the products of the bacteria, and therefore the alcohol-treated lines are able to assimilate more food, I think the fact that the increased division rate of the alcoholized lines is not permanent, but gradually declines and falls below the division rate of the control, suggests that the effect of the alcohol must be more subtle. Again the decrease in the rate of cell division due to alcohol at the early period of the cycle is not so readily explained on the assumption that alcohol is a "food" for the organisms in question.

It is obvious that the movements of the organisms in the culture medium is considerably more rapid, as a rule, when treated with alcohol. This might suggest, since food is largely received through vortex currents passing down the peristome, that more food is thus secured; but I believe that this suggestion is answered negatively by the fact that alcohol does not have a consistently accelerating action.

It might be suspected that an osmotic change brought about by the strength of alcohol used would be sufficient to influence the division rate of the infusoria, but the osmotic change is so exceedingly small that there is no reason to believe, from what is known of the effects of the phenomenon on the cleavage of eggs, etc., that any effect in this case is to be attributed to it.

As far as the experiments go I believe that they indicate that alcohol has a stimulating effect on some aspect of metabolism — possibly, as Calkins has suggested in this connection, on the secreting activities of the protoplasm. I think the evidence derived from the experiments justifies the idea that, in the case of the forms studied, alcohol supplies no "energy," so to speak, but stimulates the liberation of the "initial of potential" with which the organism is endowed.

In other words, we are justified in looking upon the protozoan cell as possessing a certain amount of metabolic energy, or it might be termed "division energy." In the normal course of the cycle, this is gradually expended in reaching the number of generations, more or less, for which the individual is endowed; but when alcohol, for example, is encountered in the environment this tends, directly or indirectly, toward a more rapid liberation of the division energy with the result that

multiplication is more rapid and more generations, for a certain length of time, are produced. But this stimulation of reproduction is not permanent, and in fact the division rate falls temporarily below the normal for the culture, as is shown by the rate of division of the control. Consequently the actual number of generations attained in the cycle is but slightly affected. From this point of view the alcohol has an effect on the individual cell of the cycle — but not on the cycle as a whole. That is, it influences the rate of reproduction but does not affect the number of generations which otherwise would be attained. This assumption will explain possibly the opposite effects produced by alcohol on the cultures at different periods of the cycle. Figs. 1 and 2 show that when the division rate is rapid the alcohol has a general depressing effect — and this may be due to the fact that the maximum division energy is being expended already, whereas when the division rate is on the decline, then the alcohol "stimulates" temporarily, and a greater number of bipartitions occur in a given period, than is the case in the control culture.

It is a point of considerable interest that alcohol produces opposite effects on the division rate at different points in the cycle, and this shows the danger of drawing conclusions from experiments on short cultures or on individuals about the ancestry of which little or nothing is known, which have been isolated merely from stock cultures. The same point is illustrated by some previously published experiments with the salts of potassium [1] — in which it was found that the dibasic potassium phosphate caused an acceleration of the rate of division during the early part of the cycle, and a retardation of the rate during the later part of the cycle. It is to be noted, however, that alcohol caused a retardation of the rate during the early part of the cycle of the *Paramecium aurelia* culture, whereas K_2HPO_4 caused an acceleration of the rate during the early part of the cycle of the *Oxytricha fallax* culture.

To draw any general conclusions from the experiments on copper sulphate at the present time would be hazardous as the results obtained, though definite, are insufficient. The data

[1] Woodruff, *loc. cit.*, pp. 617–619, Diagram IX.

show that organisms which are subjected for long periods to small amounts of alcohol, and which have attained a greater number of generations than the non-alcohol series, are more susceptible to copper sulphate; and that the lines which were subjected to the greater strength of alcohol are more susceptible to the copper sulphate than the series treated with the less strength. This shows clearly that alcohol in such amounts which may be said to be "beneficial" from the standpoint of cell metabolism, since more cell divisions have occurred, nevertheless renders the cells more susceptible to the "injurious" effects of copper sulphate. In what way this is brought about is not evident from the results obtained to date. It seems improbable that we are justified in assuming that the alcohol has caused a general "lowering of resistance" in view of the fact that the general effect of the alcohol is to increase cell division. The results suggest that probably alcohol exerts some specific effect on the metabolism of the organism, or possibly as has been suggested, for example, effects some change in the permeability of the cell membrane to copper sulphate.

VII. Summary.

The experiments briefly recorded were conducted for considerable periods on two species of Protozoa, whose status in the life-cycle was known through long cultures, and on a sufficiently large number of individuals to afford reliable averages. It is believed, therefore, that the results obtained show the general effect of alcohol on the division rate and, therefore, on the metabolism of the forms studied, when subjected to a practically constant environment.

The evidence brought forward shows that:

1. Minute doses of alcohol will decrease the rate of division at one period of the life cycle and increase it at another period of the life cycle.

2. When alcohol increases the division rate, the effect is not continuous, but gradually diminishes and finally the rate of division falls below that of the control, followed by fluctuations above and below the rate of the control.

3. An increase (doubling) of the amount of alcohol adminis-

tered, however, will again cause a more rapid cell division for a limited period. But again the effect is not constant since the rate of division falls below the control, and is followed by fluctuation above and below the division rate of the control. Up to the present time the amount of alcohol has been increased (doubled) three times, always with the same result.

4. Treatment with alcohol lowers the resistance of the organisms to copper sulphate.

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ON THE SPINNING ORGANS AND ARCHITECTURE OF EVAGRUS, A THERAPHOSID ARANEAD.[1]

C. W. STEVENSON.

The most important work on the spinning glands of spiders is that of Apstein (1892), who distinguished the five following kinds:

1. *Aciniform Glands.* — Oval-shaped glands with long duct; gland consists of tunica propria and epithelium, which stains evenly in all parts, duct of which bears no epithelium and ends on a spool with long spinning hair.

2. *Piriform Glands.* — Pear-shaped glands consisting of tunica propria and epithelium, the lower portion of which stains deeper than upper portion; the duct bears a thick tunica intima and ends on a short thick spool, with thick spinning hair.

3. *Ampullaceous Glands.* — Sac-like glands consisting of tunica propria and epithelium, of which the upper portion is cylindrical, then has a sac-like swelling from which the duct consisting of tunica propria, epithelium and tunica intima forms a double loop, the three branches of which are formed in a tunica propria, and ends on a large truncate spinning spool.

4. *Tubuliform Glands.* — Cylindrical glands consisting of tunica propria and epithelium, duct consists of tunica propria, epithelium and tunica intima and ends on a large spool.

5. *Aggregate Glands.* — Aboraceous glands of tunica propria and epithelium, with wide ramifying lumen of which the duct, consisting of tunica propria, epithelium and tunica intima bears protuberances and ends on a large spool with long pointed spinning hair.

Apstein gives only a short description of one of the Theraphosids, *Lasidora Erichsonii*, of the family Avicularíidæ. He found in these only piriform glands, of which the spinning hairs were ringed or annulated.

The only subsequent work is that of Warburton (1890) on Argiopids; McCook (1890) on Epeirids; Borgert's (1890) general

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 92.

review; and descriptions of the external anatomy of the spinnerets given by Simon (1892).

Therefore the spinning glands of Theraphosids are practically unknown and the present contribution is to present an account of a member of that group, and was done under the direction of Prof. Thos. H. Montgomery, Jr.

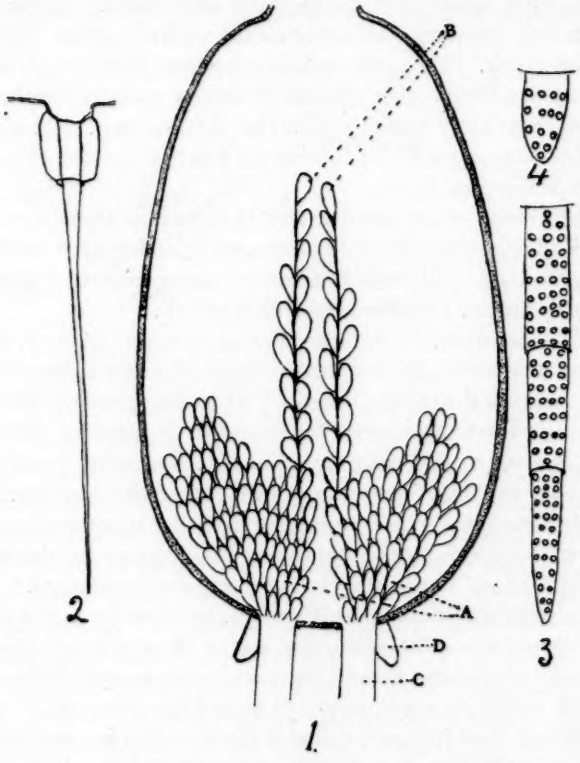
Simon (1892) divides the spiders into *Araneæ Theraphosæ* and *Araneæ Veræ*, the former including all spiders with chelicera directed forward, and comprising the families Liphistiidæ, Avicularidæ and Atypidæ. The Liphistiidæ are unique among all spiders in having four pairs of spinnerets; the Avicularidæ have only two pairs (except *Hexathele* which has three), while the Atypidæ as also most of the families of *Araneæ Veræ*, possess three pairs.

There has been a discussion as to the homologies of the two pairs of spinnerets of the Avicularidæ with the three and four pairs of the Atypidæ and Liphistiidæ. Jaworowski (1895) has shown that in Lycosids the embryonic extremities of the fourth and fifth segments of the abdomen give rise to the spinnerets in the following manner: each extremity of the fourth segment consists of two parts, endopodite and exopodite, of which the exopodites give rise to the anterior pair of spinnerets, while the endopodite of one side fuses with its fellow of the opposite side to form the colulus or its homologue the cribellum. As for the extremities of the fifth segment, the endopodites give rise to the median pair of spinnerets and the exopodites give rise to the posterior pair.

Now, it is possible, as Jaworowski believed, that of the four pairs of spinnerets of the Liphistids, the most anterior pair is homologous with the colulus and cribellum of the *Araneæ Veræ*. But just what are the homologies of the three pairs of spinnerets of the latter to the two pairs of the Avicularidæ cannot yet be decided, as the embryology of no Theraphosid has been worked out, although Simon considers the two pairs of the Avicularidæ to correspond to the anterior and median spinnerets of other spiders, while Jaworowski would hold them as homologous with the anterior and posterior spinnerets of other spiders. Accordingly in calling the spinnerets of *Evagrus* "anterior" and "posterior" respectively, following the general usage, I do not mean to prejudice the question of their homologies.

In my work on *Evagrus*, fresh material was used and the best plan was found to be to dissect them in water or very weak alcohol. The abdomen was opened from the dorsal surface, and the liver, rectum and ovaries removed, leaving the translucent spinning glands exposed to view.

There are only two pairs of glands in both males and females, one pair for each pair of spinnerets, as shown in Fig. 1. The



large pair *a*, which consists of a cluster of about 100 small piriform glands has its outlet through the larger or posterior spinnerets *c*, and the long gland *b* consisting of 12–16 piriform glands, bilaterally arranged, belongs to the smaller or anterior pair of

spinnerets *d*. The ducts of these glands end on the same kind of spools, the spools having three parts, Fig. 2, a short flexible part, then a short heavy chitinous basal piece which terminates in a long spinning hair. According to Apstein's classification, these glands would be piriform glands, being pear-shaped and ending on a spool with short thick base, but differing in having a long spinning hair.

Of the two pair of spinnerets, the posterior consists of three joints, while the anterior has only one short piece. These spinnerets bear spinning spools only on the ventral surface. On the posterior pair, Fig. 3, the spinning spools seem to be about equally distributed over the whole ventral surface, thereby differing from most spiders. On the anterior pair, Fig. 4, the number of spools seem to correspond with the number of glands which empty into it.

The females of two other genera of this same family were dissected, *Stichoplastus* (?) and *Myrmeciophila*, and these were found to agree in general with *Evagrus* in having only two pairs of spinning glands, composed of piriform glands.

In *Stichoplastus*, the glands of the posterior spinneret were similar in position and number to those of *Evagrus* but were a little larger and curved in shape. As to the glands of the anterior spinneret, they were about twelve in number, differing from *Evagrus* in that they varied in size, four being larger and far apart, and eight posterior, small and closer together. In *Myrmeciophila*, the glands were exactly similar to *Evagrus* except in number, *Myrmeciophila* possessing about 25 or 30 glands to the anterior and 150 to 200 to posterior pair of spinnerets.

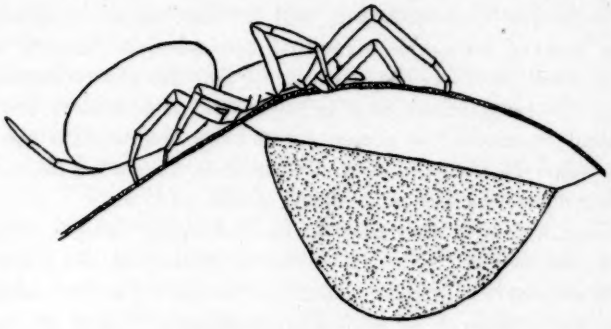
The primitiveness of these Theraphosids is shown in the limb-like elongation of the spinnerets, and in the possession of only two pairs of spinning glands, one pair to each pair of spinnerets, both of which are made up of the same kind of glands.

The only Theraphosids whose architecture has received much attention are the trap-door spiders; accordingly it may have some value to give a brief accout of the web and cocoon of *Evagrus*.

The web is always placed on the ground under large rocks, and generally in shady places where there is moisture. It seems that this species stays in colonies. Often a colony is found in one

place and fifteen feet away not a spider is to be found, from which we may conclude that the young do not scatter much. The web is a very primitive structure, containing no viscid threads and is hardly more than a thin irregular sheet woven on the ground and attached to twigs, leaves or the rock itself. There seems to be no definite form of nest but simply an irregular sheet of threads.

The cocoon of *Evagrus* is somewhat conical or cup-shaped as shown in Fig. 5, which figure also gives the relation of the



5

size of spider to that of cocoon, being a sketch of a spider only a short time after she had finished her cocoon. The cocooning process was seen only once and that time the basal piece had been woven before direct observations began. The base, which was woven first, consists of the cup-shaped lower portion. A short time after it was finished, the spider was closely observed during the remainder of the process. At 2:55 P. M. May 4, the spider placed her epigynum across this base, and discharged from her genital aperture a large yellowish drop of viscid fluid, which remained attached to her for some time, while the ova were dropping into it. At 3:15 P. M. she freed herself from this drop and immediately began to spin the cover. She did this, in which process both pairs of spinnerets were used, by standing with her legs and palpi on the margin of the base, and sweeping her spinnerets from side to side over the egg-mass, never at any

time allowing her body to touch the cocoon. Occasionally, she would change her position or take short rests. At first the threads were loosely woven, but at the latter part of the process they were closely woven. The weaving of the cover lasted from 3:19 P. M. to 3:56 P. M. Then by raising her spinnerets and swinging them from side to side above the cover, she built a kind of outside cover or what seemed later to be a suspensor above the cocoon. This lasted until 4:12 P. M. when she began biting the old connection with the web, leaving it suspended at four corners and by means of the suspensor she had just made. At 5:30 she quit operations, and left the cocoon suspended. The finished cocoon is somewhat hemispherical beneath, the base, while the cover is a flat disc. From this observation, and from observing others kept in cages, and also those found in natural conditions, the cocoon seems to be spun upon a part of the web. In the natural conditions, it is suspended from the under side of the web at about the central portion.

Here, it is seen that the cocoon of *Evagrus* consists of two parts, the base and cover. This goes to uphold the observations of Montgomery (1903), as pointed out by him for several genera, that the cocoons of all spiders are made of two parts, the base and the cover.

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ON THE SPERMATOGENESIS OF THE EARWIG ANISOLABIS MARITIMA.[1]

HARRIET RANDOLPH.

The material for the examination of the germ cells of *Anisolabis maritima* came from a colony of these earwigs at Bryn Mawr. On account of the interest in the behavior of the chromosomes in the germ cells of insects, it seems desirable to add this group to the list of those that have been investigated recently in this country.

The material was preserved in Flemming's stronger fluid or in Gilson's mercuro-nitric solution and stained by Heidenhain's iron-hæmatoxylin method or with thionin.

A preliminary paper on the spermatogenesis of *Forficula auricularia* by Zweiger, '06, which appeared in 1906, contains references to the bibliography of the subject.

In the youngest stages found at Bryn Mawr each cyst contained several spermatogonia. In these cells (Fig. 1) in the resting stage there is a large spherical body which stains like chromatin.

In the equatorial plate of the dividing spermatogonia seen in polar view there are twenty-four chromosomes and a plasmosome which stains faintly (Fig. 2).

In the telophase of the last spermatogonial division two chromosome rods become connected with the plasmosome and remain condensed throughout the growth stages of the first spermatocytes.

Synizesis and synapsis stages are shown in Figs. 3 and 4, and the spireme in Fig. 5. At some time during the growth stages of the spermatocyte the heterochromosome pair separate from the plasmosome, forming a single rounded mass which lies free in the nuclear space (Figs. 6 and 7).

The splitting of the chromosomes is shown in Fig. 8. In Fig. 9 chromosomes from the prophase of the first spermatocyte are

[1] For the identification of the species I am indebted to the kindness of Mr. J. A. G. Rehn, of the Academy of Natural Sciences, Philadelphia, Penna.

shown, together with the heterochromosome pair and the plasmosome. All are from the same cyst. Figs. 10, 11 and 12 are from late prophases of the first spermatocyte division. The chromosomes arrange themselves into two groups at opposite poles. This is shown in Fig. 10, where the black bodies represent one group and those in light outline are 180° away, while the one in heavy outline is approximately at the equator. Figs. 11 and 12 show a centrosome close to each group which had apparently moved with its centrosome to that position. That the final position of any chromosome at one or the other of the poles is due to the centrosome in whose sphere of influence it happens to lie is suggested by the fact that occasionally seven chromosomes are at one pole and only five at the other.

In the equatorial plate of the first spermatocyte division there are normally twelve chromosomes (Fig. 13). In two or three earwigs a few cells of a cyst show eleven, thirteen, sixteen or nineteen chromosomes in the equatorial plate of this division; but there are also present in these cysts tripolar or multipolar spindles which probably explain the irregularity. There are also occasionally giant nuclei with double the normal number of chromosomes. In one case of an abnormal spindle it is known that the material came from an earwig that had very recently moulted, and it is possible that there is a connection between the two facts (Riddle, '08). In one instance a tripolar spindle was observed also in a spermatogonial division.

In the anaphase of the first spermatocyte division the heterochromosome pair are late in dividing and lag behind the others (Figs. 15, 16, 17). They are about equal in size. They finally separate (Figs. 18, 19, 20), one going to each pole of the spindle, and pass into the prophase of the second spermatocyte division (Fig. 21). Here again the chromosomes show a tendency to arrange themselves at the poles. Fig. 22 shows the metaphase and Fig. 23 the equatorial plate of the second spermatocyte division with twelve chromosomes. Figs. 24 and 25 are from stages in the anaphase. The earliest stages of the spermatids are shown in Figs. 26, 27, 28 and 29, where the behavior of the archoplasm and the change in position of the centrosome can be seen. Fig. 32 shows the condensed chromatin body in

the young spermatozoa. The older spermatozoa arrange themselves in bundles by inserting their heads into a cyst cell.

The material which is to form the spindle fibers is conspicuous at an early stage and is very considerable in amount. It forms another layer of fibers around the spindle proper (Figs. 5, 21, 22).

Something like the "mitosoma" described for *Forficula* by Zweiger, '06, is present in *Anisolabis* (Fig. 1), although the form is apparently unlike in the two species. It is traceable possibly from the spermatogonium to the spermatid; but as it does not stain with thionin after an early stage, and as it is very small and there are many granules in the iron-hæmatoxylin preparation, it is not by any means certain that the structures observed are one and the same throughout the series (Fig. 20).

The somatic chromosome number, found in the cells of the egg follicle, is twenty-four (Fig. 30). The material was not favorable for the examination of the ova. Very few were found in division stages and only one was cut so that its chromosomes could be counted. In this only equatorial plate observed the number of the chromosomes is twenty-four.

The characteristic structure of the male germ cells of *Anisolabis maritima* is an equal heterochromosome pair which are present possibly in the spermatogonia although they are not distinguishable from the other chromosomes in the spermatogonial divisions. In any case, it is formed anew in the telophase of the last spermatogonial division. It remains condensed during the growth stages of the first spermatocyte and divides equally in the first spermatocyte division, lagging behind the other chromosomes in the anaphase. It is not evident in the second spermatocyte division but there is a condensed chromatin body in the spermatids. This equal heterochromosome pair appears to be like the equal pair of idiochromosomes found by Wilson, '05, in *Nezara* and the equal heterochromosome pair of Stevens, '06, in Lepidoptera.

Bryn Mawr College,
June 2, 1908.

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Explanation of Plate I.

All figures were drawn with Zeiss camera lucida, 2 mm. oil immersion objective, 12 ocular, enlarged 2 diameters with a drawing camera and reduced the same.

Fig. 1. Spermatogonium, resting stage. *m* (?), the "mitosoma" of Zweiger.

Fig. 2. Spermatogonium, equatorial plate. *p*, the plasmosome.

Fig. 3. First spermatocyte, synizesis stage. *hc*, the heterochromosome.

Fig. 4. First spermatocyte, synapsis stage.

Fig. 5. First spermatocyte, spireme stage.

Fig. 6. First spermatocyte, the heterochromosome pair partially separated from the plasmosome.

Fig. 7. First spermatocyte, the heterochromosome pair separated from the plasmosome.

Fig. 8. First spermatocyte, the chromosomes partially split.

Fig. 9. First spermatocyte, prophase; the forms of the chromosomes, all from the same cyst.

Fig. 10. First spermatocyte, prophase; the six chromosomes in solid black are at one pole and the five in outline at the opposite pole; the one in heavy outline is at the equator.

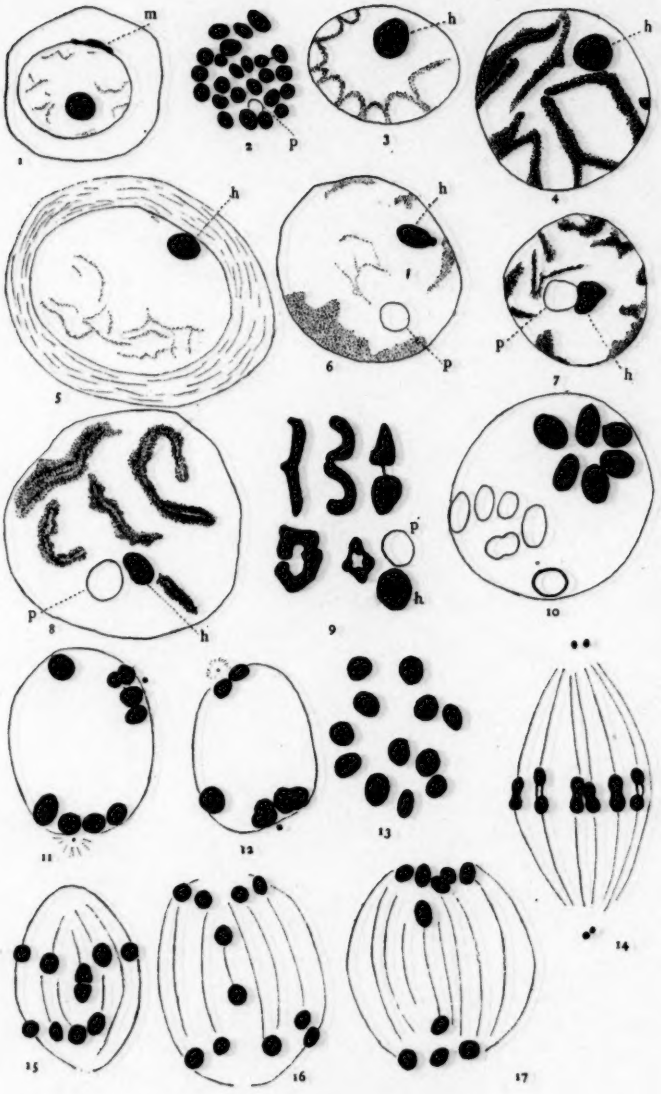
Figs. 11 and 12. First spermatocyte, prophase; the chromosomes on the way to opposite poles under the influence of the centrosomes.

Fig. 13. First spermatocyte, equatorial plate.

Fig. 14. First spermatocyte, metaphase.

Fig. 15. First spermatocyte, early anaphase.

Figs. 16 and 17. Later anaphase stages.


m
h
p
1
2
3
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Explanation of Plate II.

Figs. 18 and 19. Late anaphase stages.

Fig. 20. Telophase; *?m*, the "mitosoma" of Zweiger.

Fig. 21. Second spermatocyte, prophase.

Fig. 22. Second spermatocyte, metaphase.

Fig. 23. Second spermatocyte, equatorial plate.

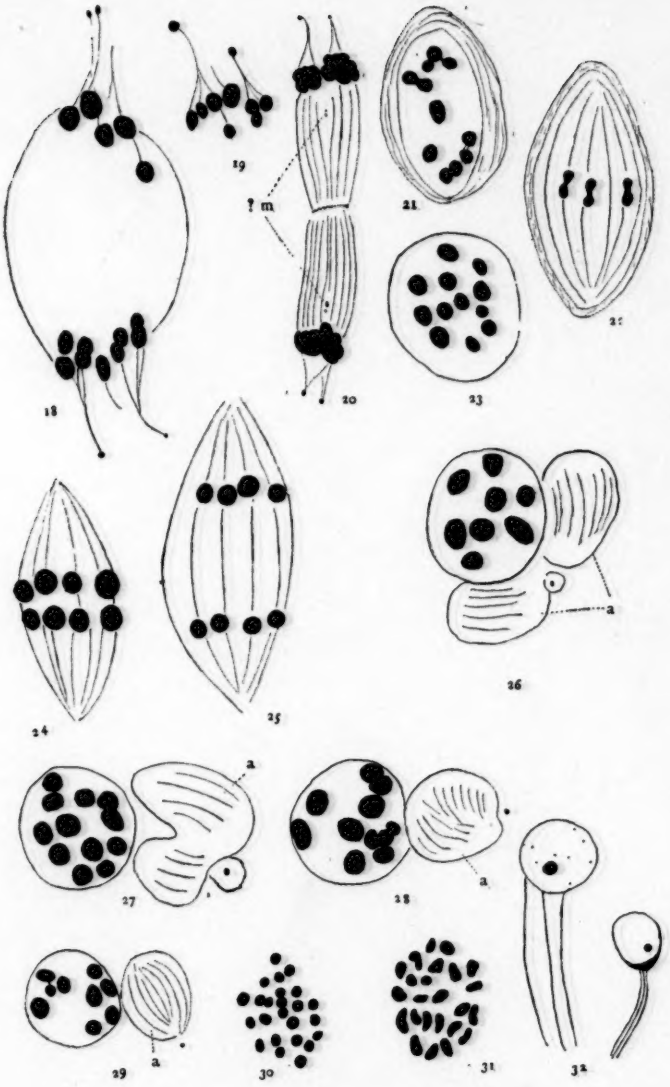
Figs. 24 and 25. Second spermatocyte, anaphase stages.

Figs. 26, 27, 28, 29. Spermatids, showing archoplasma (*a*) in different stages, and the movement of the centrosome.

Fig. 30. Somatic cell of female, equatorial plate.

Fig. 31. Ovum, equatorial plate.

Fig. 32. Young spermatozoa containing condensed chromatin.


18
19
? m
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21
22
23
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25
26
a
27
a
28
a
29
a
30
31
32

Species.	Length in mm.	Width in Per Cent. of Length.	Lat. Field.		Muscle.				Diminution at Septum.			Position of Greatest Width.				Tail Length in Per Cent. of Total Length.	Corona Ciliata.			Collarette Present.	Collarette Absent.	General Appearance.				Number of Anterior Teeth
			Large	Small.	Strong.	Weak.	Broad.	Narrow.	Sudden.	Gradual.	None.	At Center.	Behind Center.	In Front Center.	Evenly Wide.		On Head.	On Body.	On Both.			Opaque.	Transparent.	Firm.	Flaccid.	
S. bedoti.....	5	6	×		×			×	×					×		22			×		×	×		×		9–1
	18															30										
S. enflata.....	9	9	×			×				×		×				16	×				×		×		×	7–1
	29	10														25										
S. ferox.....	10	5.8		×	×				×						×	26	×			×		×		×		6–1
	20															36										
S. hexaptera.....	15	10	×			×	×		×			×				17	×				×		×		×	1–1
	47															26										
S. macrocephala..	7	7.6		×						×		×				28										
	18															40			×		×			×		5–1
S. neglecta.....	5	5		?	×		×			×	?			×		26		×		×		×		×		3–7
	10	6.5														40										
S. bipunctata.....	9	6.3		×		×	×			×				×		21		×			×	×		×		4–7
	20															25										
S. regularis.....	4	6		?	×		×				×				×	28		×		×		×		×		2–4
	27															40										
S. pulchra.....	9	10.5	×			×			×			?				18			×	×			×		×		5–1
	22															27										
S. robusta.....	10	4		×	×					×					×	25				×		×		×		5–1
	14	5.7														33										
S. serratodentata.	5	5		×		×	×			×		×				22			×		×	×		×		3–7
	15															36										
S. zetesios.....	9	6.3		×	×		×			×		×				25				×		×		×		4–9
	32															33										
S. furcata.....	12	10	×			×	×		×					×		17	×				×		×		×	4–8
	35															25										
S. siboga.....	9	6.5	×		×		×		×					×		21				×		×		×		7–1
	20															33										
S. minima.....	10			?												16.5										3–4
S. decipiens.....	6	5								×			×			25				×			×	×		5–1
	12															40										
S. planctonis.....	20	10	×						×							25		×			×					5–6
S. whartoni.....	30	6								×		×				20					×					3–5
	45	11														26										

Issued February 20, 1908.
Publication postponed to article by E. L. Kelly published July, 1908, and should face page 80.

TABLE OF CHARACTERS.

ral ance.				Anterior Fin.									Posterior Fin.											
Firm.	Flaccid.	Number of Anterior Teeth	Number of Posterior Teeth.	Length in Per Cent. of Total Length	Width in Per Cent. of Total Length.	Extends to Ventral Ganglion.	Remote from Ventral Ganglion.	Approaching Ventral Ganglion.	Widest at Center.	Widest in Front of Center.	Widest Behind Center.	Distance in Per Cent. of Total Length from Anterior to Posterior Fins.	Length in Per Cent. of Total Length.	Width in Per Cent. of Total Length.	Extends to Seminal Vesicle.	Not to Seminal Vesicles.	Longer Than Anterior Fin.	Shorter Than Anterior Fin.	Same as Anterior Fin.	Widest at Tail Septum.	Widest in Front of Tail Septum.	Widest Behind Tail Septum.	More Than 50 Per Cent. in Front of Tail Septum.	Less Than 50 Per Cent. in Front of Tail Septum.	Tooth Row Longer.
×		9–13	20–32	20	2.4			×			×	5.4	24	4.5	×				×			×		47	
	×	7–10	12–17	11–17	1.5 2.8		×		×			6.8	17+	4.5		×			×	×			66 75		
×		6–10	10–14	28–30	4 4.6	×					×	5.7 6.8	23+	4+	×			×				×		40+	
	×	1–4	1–6	8.6 13	1.7 2.3		×		?		×	11.1	17+	4.3		×	×			×			51 60		
×		5–10	14–32	10.7	2.6						×	7.5	16+	3.9	?		×			×			50+		×
×		3–7	9–15	18–21	1.2 2.5	×					×	6.5	23+	4.1	×		×					×		35 45	
×		4–7	8–18	15.9	1.5		×			×		7.9	17+	2.7		×	×			×			68 75		
×		2–4	2–6	13+	1.9			×	×			6.8	23+	2.0	×				×			×		33+	
	×	5–10	9–19	34.6	3.8	×					×	5.7	24—	6.2	×			×		×		?	64		
×		5–10	11–14	20	2.9			×			×	6.8	26--	3.7	×		×					×		35	
×		3–7	8–13	20–24	3.0	×					×	7.5	25	4.4	×		×					×	52		
×		4–9	8–19	22	2.6			×			×	9.5	15	3.0		×		×		×	?	?	62		×
	×	4–8	5–12	25	2.4			×			×	6.1	20	4.5		×			×		×		70		
×		7–11	13 23	25	1.4 1.8	×					×	15	15	3.2		×		×		×			50		
		3–4	7–8					×																	
×		5–10	12–18	11.5	2.5			×				8.5	19	4.9					×	×		?	56		
		5–6	12	19	5	×			×			10	19	6.2			×			×		?	67		
		3–5	5–7	44–66	2.8	?			×			0.0	19–33	5.7				×				×		40 42	

× denotes presence of character. ? possibly present but not observed.

RS.

Less Than 50 Per Cent. in Front of Tail Septum.	Vestibular Ridge.						Seizing Jaws.																					
								Shaft.									Point.							Pulp.					
	Tooth Row Longer.	Tooth Row Shorter.	Lateral Process Blunt.	Lateral Process Acute.	Acute Papillæ.	Blunt Papillæ.	Number.	Greatly Curved.	Slightly Curved.	Serrated.	Not Serrated.	Crested.	Not Crested.	Cross-Section Oval.	Cross-Section Triangular.	Pin Shaped.	Slightly Curved at Apex.	Broadly Conical.	Sickle-Shaped.	Per Cent. in the Shaft.	Base and Top of Shaft Converge at Back.	Base and Shaft Top Diverge at Back.	At Focus.	At Center.	Between the Two.	Swollen Near Base of Point.	Bent Near Base of Point.	Straight.	Canal Scattered.	Canal Concentrated.
47				×	×		5–7		×		×																			
			×			×	7–10	×			×	×	×	×			×			16			×					×	×	
40+			×			×	5–6	×			×									20										
			×				5–13	×			×	×			×	×		×		25		×			×		×	×		
	×	×					9–12	×			×																			
35							5–8		×		×																			
45		×	×			×	8–10	×			×		×	×		×				33			×					×	×	
33+							6–9		×		×																			
				×	×		5–7	×			×																			
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	×					×	8–10	×			×																			
							6–9	×			×	×	×		×		×			16										
					×															20	×				×					×
							5–7		×		×																			
							6–8	× at tip.			×	×	×		×				×	16					×	×			×	
		×		×			5–6		×		×		×																	
							8		?		×																			
40		×																												
42							8–10	×																						